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43885	Children's Can. Res. Fnd. Weiner, R.	Improvement of Assays for Cell Mediated Immunity: Methods for Isolation & Preservation of Lymphocytes for Reproducible Reactivity in Immunological Tests	0-57
23877	City of Hope Nat'l Med.Ctr. Todd, C. W.	Purified Carcinoembryonic Antigen and Antisera	0-58
43887	Emory Univ. Hersh, T.	Evaluation of Assays for Circulating Tumor Associated Antigens: Clinical Usefulness of Carcinoembryonic Antigen (CEA) Assay as an Adjunct in the Diagnosis of Gastrointestinal Cancer	0-59
43888	Emory Univ. Vogel, C. L.	Evaluation of Assays of Circulating Tumor Associated Antigens: Clinical Usefulness of the Alphafetoprotein (AFP) Test and Human Chorionic Gonadotropin (HCG) in the Differential Diagnosis of Testicular Cancer	0-60
92176	George Wash. Univ. Hollinshead, Ariel	Cutaneous Hypersensitivity Antigen Reactions	0-61

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43891	Iowa Univ. Osborne, J. W.	Development of Animal Models for Circulating Tumor Associated Antigens Potentially Useful in the Immunodiagnosis of Carcinoma	0-62
43965	Johns Hopkins Univ. Henney, C. S.	Improvements of Assays for Cell Mediated Immunity: Development of New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells	0-63
33869	Kentucky Univ. Goldenberg, D. M.	Experimental Systems for Carcinoembryonic Antigen	0-64
43886	Kobe Univ. Kobata, A.	Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins	0-65
43953	Mt. Sinai Sch. of Med. Paronetto, F.	Detection by Immunoflourexcent Techniques of Antibodies Against Autologous Human Tumor Cells	0-66
43889	Massachusetts Gen. Hospital Alpert, E.	Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcinoembryonic Antigen and Alphafetoprotein	0-67
23879	Mayo Found. Wahner, H.	Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests	0-68
33875	Meloy Labs. Woods, R.	Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine	0-69
43861	Miami Univ. Rosenberg, E. B.	Procurement of Tumor Specimens and Serial Serum Specimens from Cancer Patients	0-70
33915	Minnesota U. Najarian, J. S.	Collection of Sera from Populations with High Cancer Risk	0-71
43853	Minnesota U. Yunis, E. J.	Assays for Immune Depression in Cancer Patients	0-72

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33914	Philadelphia Geriatric Ctr. Altschuler, H.	Collection of Sera from Populations with High Cancer Risk	0-73
43858	Robert B. Brigham Hosp. Schur, P. H.	Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcioembryonic Antigen and Alphafetoprotein	0-74
33858	Roswell Park Mem. Inst. Ming Chu, Tsann	Carcinoembryonic Antigen and Related Tumor-Specific Antigens in the Diagnosis of Cancer and as an Adjuvant in the Management of the Cancer Patient	0-75
43854	Sloan Kettering Inst. for Cancer Res. Fogh, J. E.	Isolation and Tissue Culture of Human Tumor Cells	0-76
43881	Southern Cal. Univ. Friou, G. J.	Detection by Immunoflourescent Techniques of Antibodies Against Autologous Human Tumor Cells	0-77
43989	State Univ. of New York Bahl, Om	Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins	0-78
43893	Texas Med. School Harrington, J. T.	Improvements of Assays for Cell Mediated Immunity: Development of New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells	0-79
43883	Uppsala U. Wigzell, H.	Isolation and Characterization of Human Peripheral Blood Mononuclear Cells	0-80
43890	West Virginia Univ. Veltri, R. W.	Search for New Human Tumor Associated Antigens in Carcinoma of the Lung	0-81
43882	Wistar Inst. Manson, L.	Development of Animal Models: Early Detection of Antibodies to Carcinomas in Experimental Animals as a Model for Human Studies	0-82

Immunology Contract Program (Cont'd)

43859	Yale Univ. Marchesi, V.	Biochemistry and Diagnostic Use of Human Tumor Antigens	0-83
GLC(70)-53	Armed Forces Radiobiolog. Res. Inst. Kiker, W.	Irradiate Experimental Animals	0-84
43849	Boston Univ. Med. School Deckers, P. J.	Subcellular Fractions in Immunotherapy - Augmentation of Tumor-Specific Immunity with Ribonucleic Acid (RNA)	0-85
43875	California Inst. of Tech. Dreyer, W.	Development of Immunolatex Particles for Detection of T and B Cells	0-86
43852	California U. L. A. Morton, D. L.	Human Melanoma: Evaluation of BCG Immunotherapy of Patients without Detectable Disease after Removal of Tumor-Containing Lymph Nodes	0-87
43876	Childrens Hosp. Med.Ctr. Parkman, R.	Hybrid Tumor Cells: An Immunotherapeutic Agent	0-88
43857	Florida Univ. Waldman, R. H.	Human Lung Cancer: Evaluation of BCG Therapy	0-89
43950	Harbor Gen. Hospital Pilch, Y. H.	Subcellular Fractions in Immunotherapy	0-90
02208	Lautenberg Ctr. for Gen. & Tumor Immu. Hebrew U. Weiss, D. W.	Studies on Methanol Extractable Residue (MER) of BCG	0-91
43872	Jewish Hosp. of St. Louis Little, J. R.	A Study of the Immunological Stimulant Properties of Amphotericin B	0-92
43954	Lincoln Labs. Hein, G. L.	Multi-test Device with Standardized Antigens to Assay Delayed Hypersensitivity Via the Skin Test	0-93
02103	Litton-Bionetics, Inc. Darrow, C.	Conduct Bone Marrow Transplantation Studies	0-94

Immunology Contract Program (Cont'd)

23227	Litton Bionetics, Inc. McCoy, J.	Measurement of Immunological Reactivity to Human Cancer	0-95
43946	Minnesota U. McKhann, C. F.	Immunotherapy: Role of Circulating Tumor Antigens	0-96
92061	Minnesota U. McKhann, C. F.	Tumor Specific Transplantation Antigens in Solid Tumors	0-97
43948	Minnesota U. Schmidtke, J. R./ Nelson, R. D.	Role of Macrophages in Tumor Immunology	0-98
23885	Minnesota U. Simmons, R. L.	Immunotherapy of Cancer	0-99
33853	Mt. Sinai Med. Center Bortin, M. M.	Graft Versus Tumor: A Model for the Treatment of Carcinoma, Leukemia and Lymphoma	0-100
43879	Mt. Sinai Sch. of Medicine Holland, J. F. Bekesi, J. G.	Chemoimmunotherapy of Acute Myelocytic Leukemia	0-101
43878	Ohio State U. LoBuglio, A. S.	Transfer Factor Therapy of Sarcoma	0-102
43864	Oklahoma Med. Res. Found. Kollmorgen, G. M.	Immunotherapy: Development of Animal Models for Evaluation of Therapy with Neuraminidase Treated Tumor Cells	0-103
43873	Palo Alto Med. Res. Fnd. Krahenbuhl, J.L.	Studies on the Role of Macrophages in the Immune Response	0-104
33891	M. S. Hershey Med. Center Bartlett, G. L.	Cancer Immunotherapy: Animal Models for Treatment of Minimal Residual Systemic Tumor	0-105
43967	Pennsylvania Univ. Arai, K.	Hapten Treatment of Cancer	0-106
33860	Pennsylvania Univ. Gasic, G. J.	Neuraminidase Effectiveness in Tumor Immunotherapy and Mechanism of Its Effect	0-107

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33373	Research Fnd. of the State of New York Klein, E.	Immunotherapy of Accessible Neoplasms: An Analysis of Immune Status In Vivo	0-108
33896	Robert B. Brigham Hosp. Churchill, W. H.	Macrophage Activation in Tumor Immunity and Immunotherapy of Rat Mammary Tumor	0-109
43951	Rush-Presbyterian-St. Luke's Med. Center Baram, P.	Subcellular Fractions and Immunotherapy: Effect of Transfer Factor on Virus-Induced Tumors in Marmoset Monkeys	0-110
43874	Scripps Clinic and Res. Fnd. Feldman, J. D.	Studies on the Role of Circulating Tumor Antigens in Immunotherapy	0-111
33888	Texas Univ. M. D. Anderson Hosp. Gutterman, J. U.	Immunotherapy of Human Cancer	0-112
23887	Washington U. Hellstrom, K. E.	Immunotherapeutical Trials with Human Tumors	0-113
43877	Virginia Med. College Kaplan, A. M.	Immunotherapy: Mechanism of Action of Immunopotentiators	0-114
43865	Yale Univ. Gershon, R. K.	Development of Animal Model for Evaluation of Therapy with Neuraminidase Treated Tumor Cells	0-115

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43897	Baylor College of Medicine Horning, E. C.	Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer	P-12
23870	Boston City Hospital Monaco, A.	Development of Immunological Assays for Human Breast Cancer	P-13
33882	California U. San Francisco Petrakis, N. L.	Analysis of Breast Secretions	P-14
23871	Albert Einstein College of Med. Marcus, D.	Purification of an Antigen from Human Mammary Carcinoma	P-15
23872	Albert Einstein College of Med. Baum, G.	Ultrasound Mammography	P-16
23230	Henry Ford Hosp. Talley, R. W.	Primary Breast Cancer Patient Input-Follow-up	P-17
23868	Institute for Med. Research Lasfargues, E.	Tissue Culture Lines from Patients with Breast Cancer	P-18
50573	Louisiana State Univ. McMahan, C. A.	Experimental Design and Statistical Services for Studies in the Treatment of Primary Breast Cancer	P-19
23231	Downstate Med. Center(SUNY) Carter, A. C.	Primary Breast Cancer Study Patient Input (Follow-up)	P-20
43869	Oklahoma Health Sciences Center Haberman, J. D.	Mass Screening for Breast Cancer Electronic Infrared Pattern Recognition	P-21

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33904	Pittsburgh Univ. Sashin, D.	Electronic Mammography	P-22
71465	Research Fnd. for Mental Hygiene Vestergaard, P.	Steroid Excretion Study in Patients with Breast Cancer	P-23
80704	St. Vincent Hosp. Friedell, G.	Pathology Laboratory for Cooperative Clinical Studies	P-24
23867	Medical College of So. Carolina O'Brien, P.	Breast Cancer Tissue Supply	P-25
23869	M. D. Anderson Hospital Reeves, W. and Cailleau, R.	Tissue Culture Lines from Patients with Breast Cancer	P-26
New Contract	Duke University Lester, R.	Xeromammography vs Film Mammography - A Comparative Study	P-27
43871	Vanderbilt U. Page, D. L.	Fibrocystic Disease of the Female Breast and its Relationship to Mammary Carcinoma	P-28
23232	Vermont Univ. Haines, C. R.	Primary Breast Cancer Study Patient Input (Follow-up)	P-29
43898	Worcester Fnd. for Exper. Biology, Inc. Welsch, F.	Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer	P-30
60968	Worcester Fnd. for Exper. Biology Welsch, F.	Automated Methods for Determination of Steroid Metabolites	P-31
33901	Creighton U. Lynch, H. T.	Breast Cancer Family Resources	P-32
New Contract	Creighton U. School of Med. Lynch, H. T.	Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families	P-33

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02076	International Agency for Res. on Cancer Tulinius, H. & Bajarnason, O.	A Study on Familiality of Breast Cancer in Iceland	P-34
33921	Kaiser Fnd. Res. Inst. Kodlin, D.	Epidemiological and Etiological Characteristics of Chronic Cystic Mastopathy and Relationship to Breast Cancer	P-35
New Contract	Montefiore Hosp. & Med. Ctr. Fishman, J.	Biochemical and Physiological Investigations Based on Familial Genetic Patterns	P-36
New Contract	The Netherlands Cancer Inst. Cleton, F. J.	Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families	P-37
33885	Queen Wilhelmina Fund deWaard, F.	Role of Diet and Nutritional Status on Incidence, Pathogenesis and Natural History of Breast Cancer	P-38
New Contract	Texas Univ. System Cancer Center Anderson, D. E.	Biochemical and Physiological Investigations Based on Familial Genetic Patterns	P-39
New Contract	Texas Univ. System Cancer Center Anderson, D. E.	Investigations of Genetic Polymorphisms in High and Low Risk Breast Cancer Families	P-40
33902	Worcester Fnd. for Exper. Biology Longcope, C.	Breast Cancer and Estriol Dynamics	P-41
New Contract	Baylor College of Medicine Kohler, P. O.	Role of the Stroma in the Growth of Neoplastic and Preneoplastic Lesions of the Mammary Gland	P-42
New Contract	California U. Berkeley Cole, R. D.	Mammary Gland Responsiveness to Multiple Hormones	P-43
23861	California U. San Francisco Elias, J. J.	Hormonal Requirements of Normal and Abnormal Human Mammary Tissue in Organ Culture	P-44

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90033	California Univ. Davis, Calif. Faulkin, L. J.	Study of Mammary Tumors in Dogs	P-45
81461	California Univ. Davis, Calif. Wellings, S. R.	Human Mammary Dysplasia Study	P-46
New Contract	California Univ. Medical School Davis, Calif. Wellings, S. R.	Neoplastic Potential of "Preneoplastic Lesions of the Human Mammary Gland"	P-47
23859	Case Western Reserve Univ. Pearson, O.	Role of Prolactin in Breast Cancer	P-48
23873	Chicago Univ. DeSombre, E.	Biochemical Nature of Hormone Dependency in Breast Cancer	P-49
33906	Children's Hospital Oakland, Cal. Abraham, S.	Identification of Cell Types in Cultures of Normal and Neoplastic Breast Tissues	P-50
33907	City of Hope Med. Center Ohno, S.	Studies to Determine the Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice	P-51
23857	Duke Univ. McCarty, K. S.	Protein Nucleic Acid Interactions in Transcription Controls of Normal and Malignant Cells	P-52
33909	Georgia Med. College Scott, D. F.	Studies on the Role of 3', 5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia	P-53
23856	Illinois Univ. Larson, B. L.	Cellular Control in Mammary Involution and Dedifferentiation	P-54
33883	Institute for Molecular Biol. Kratochwil, K.	The Response of the Embryonic Mammary Gland to Androgenic Hormones	P-55
New Contract	Sloan-Kettering Insti. for Cancer Research Bendich, A.	Effects of Nucleic Acid Preparations on the Biological Properties of Mammary Carcinomas	P-56

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31146	Mason Res. Inst. Bogden, A. E.	Biological Characterization of Animal Mammary Tumors and Tumor Bank Facility	P-57
33918	Massachusetts General Hosp. Robinson, D. R.	Studies on the Role of Prostaglandins in Mammary Gland Neoplasia	P-58
33912	Mt. Sinai School of Med. Rubin, A.	Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and Influence Cellular Biochemistry	P-59
33910	Oklahoma State University Ebner, K. E.	Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry	P-60
23858	Pennsylvania Medical College Leighton, J.	Diagnostic Characterization of Mammary Tumors	P-61
33898	Pennsylvania State Univ. Gaffney, E. V.	Cultivation of Normal and Malignent Human Mammary Tissue: Hormone Dependent Susceptibility of Mammary Tissue to Transformation	P-62
23863	Pennsylvania State Univ. Hymer, W. C.	Isolation of Prolactin Cells from Human and Rat Adenohypophysis; A New Approach to the Study of Control of Prolactin Secretion in Relation to Mammary Cancer	P-63
33880	Rochester Univ. Hilf, R.	Studies of Isoenzymes in Normal, Pre-Neoplastic and Neoplastic Breast Tissues as an Approach to Define Unique Properties of Neoplasia	P-64
23864	Roswell Park Memorial Inst. Kim, U.	Studies on the Prevention of Metastasis in Mammary Cancer	P-65
23865	Roswell Park Memorial Inst. Dao, T. L.	Studies on Basic Biological Events in the Pathogenesis of Mammary Cancer	P-66
23866	Roswell Park Memorial Inst.	The *In Vitro* Cell and Mammary Cancer	P-67

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New Contract	Scripps Clinic and Res. Fnd. Dandliker, W. B.	Mammary Gland Responsiveness to Multiple Hormones	P-68
23860	Southern California U., L.A. Sherwin, R. P.	Documentation of Human Breast in Tissue Culture and the Recognition of Growth Patterns	P-69
23875	Stanford Univ. Stockdale, F. E.	Control of DNA Synthesis in the Mammary Gland	P-70
33905	Leland Stanford Jr. University Lucas, Z.	Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry	P-71
33856	Stehlin Found. Giovanella, B.C.	Study Breast Cancer Transplantability in Nude-Athymic Mice	P-72
New Contract	Texas Univ. Medical Br. Abell, C. W.	Growth Alteration of Mammary Neoplastic Cells Obtained by Manipulation of Cellular Environment	P-73
23862	Texas Univ. Medical School McGuire, W. L.	Biochemical Mechanism of Endocrine Induced Breast Cancer Regression	P-74
33919	Worcester Fnd. for Exper. Biol. Maudsley, D.	Studies on the Role of 3', 5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia	P-75
33920	Worcester Fnd. for Exper. Biol. Burstein, S.	Studies on Role of Prostaglandins in Mammary Gland Neoplasia	P-76
23874	Worcester Fnd. for Exper. Biol. Fairbanks, G.	Intracellular Membrane Biogenesis in Hormone-Medicated Differentiation of Mouse Mammary Epithelium In Vitro	P-77
43899	Allegheny Gen. Hospital Schiffer, L. M.	Cell Kinetics of Breast Cancer	P-78

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New Contract	California Univ. at Los Angeles Sparks, F. C.	Therapy of Patients with Stage II and Stage III Carcinoma of the Breast	P-79
New Contract	Case Western Reserve Univ. Hubay, C.	Therapy of Patients with Stage II and Stage III Carcinoma of the Breast	P-80
60945	Chicago Univ. Jensen, E.	Develop a Method for Predicting Response to Adrenalectomy	P-81
New Contract	Emory Univ. School of Med. Vogel, C.	Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients	P-82
43900	Health Res.Inc. Roswell Pk Div N.Y. St. Dept. of Health Dao, T.	Investigation of Steroid Sulfational Estrogen-Binding in Human Breast Cancer	P-83
43914	Mason Res. Inst. Bogden, A. E.	Studies in Predictive Transplantable Animal Mammary Tumor Models	P-84
33899	Mayo Clinic Ahmann, D. L.	An Evaluation of Surgical Adjuvant Chemotherapy Utilizing 5-Fluorouracil, Cytoxan and Prednisone in Patients with Breast Cancer	P-85
33903	Goldwater Mem. Hospital (NYU) Post, J.	Cell Kinetics of Breast Cancer	P-86
33861	Papanicolaou Cancer Res.Inst. Zucker, R. M.	New Techniques in Cell Kinetics of Breast Cancer	P-87
23876	Pittsburgh Univ. Fisher, B.	Primary Breast Cancer Group (NSABP)	P-88
33886	Rush-Presbyterian-St. Luke's Medical Center Sky-Peck, H. H.	Cell Kinetics of Breast Cancer	P-89

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New Contract	Rush-Presbyterian St. Luke's Medical Center Perlia, C.	Study Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients	P-90
33881	Scripps Clinic and Research Fnd. VanderLaan, W. P.	Prolactin Radioimmunoassays in Human Serum	P-91
43867	Worcester Found. for Exper. Biol. Welsch, F.	Estrophile Binding and Estrophile Proteins in Human Breast Cancer	P-92

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Contract Research Summaries:

Contract No. NO1-CN-	Institution Investigator	Title	
New Contract	Arizona Med. Ctr. Arizona Univ. Present, A. J.	Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy	R-4
New Contract	Baylor College of Medicine Hazlewood, C.F.	Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues	R-5
New Contract	California U. USAEC, Lawrence Berkeley Lab	Exploration of the Use of a Proton Beam in Tissue Densitometry	R-6
New Contract	Chicago Univ. Moosa, A. R.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis	R-7
New Contract	Chicago Univ. Skaggs, L. S.	Exploration of the Use of a Proton Beam in Tissue Densitometry	R-8
New Contract	Cincinnati Med. Ctr. Buncher, C. R.	Statistical Center for Cooperative Lung Cancer Groups	R-9
New Contract	Columbia Univ. Hilal, S. K.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	R-10
New Contract	Cornell Univ. Med College Potts, D. G.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	R-11
New Contract	Duke Univ. Nefzger, M.D.	Conduct of a Tumor Registry for Patients who have had Extensive X-Radiation or Chemotherapy	R-12
New Contract	Hahnemann Med. College and Hospital Brady, L. W.	Clinical Application and Evaluation of "Xonics" Electron Radiography	R-13

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New Contract	Prof. Staff Assoc. Harbor Gen. Hospital Odell, W. D.	Hormone Markers for the Detection and Diagnosis of Cancer	R-14
35-21	Health Insur. Plan of Greater New York Fink, R.	Evaluation of Thermography in Mass Screening for Breast Cancer	R-15
35027	Thomas Jefferson Univ. Wallace, J. D.	Evaluation of Thermography in Mass Screening for Breast Cancer	R-16
92172	Johns Hopkins Hospital Frost, J. K.	Lung Cancer - Early Detection, Localization and Therapy	R-17
New Contract	Johns Hopkins Univ. Hollis, D. P.	Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues	R-18
New Contract	Memorial Hosp. for Cancer & Allied Diseases Fitzgerald, P.J.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis	R-19
New Contract	Memorial Hosp. for Cancer & Allied Diseases Melamed, M. R.	Lung Cancer - Early Detection, Localization and Therapy	R-20
New Contract	Louisville Univ. Foundation, Inc. Songster, C. L.	Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or more (over 7-10 mls per 24 hrs) of Human Blood in Human Feces	R-21
New Contract	Massachusetts General Hosp. New, P. F. J.	Clinical Evaluation of the Use Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	R-22
New Contract	Mayo Foundation Baker, H. L.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	R-23
22000	Mayo Clinic Fontana, R. S.	Detection and Localization of Bronchogenic Cancer	R-24

Cancer Diagnosis Contract Program (Cont'd)

23854	Mayo Clinic Go, V. L. W.	Carcinoembryonic Antigen (CEA) Test, A New Method and Its Evaluation	R-25
New Contract	Mayo Foundation Go, V. L. W.	Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis	R-26
New Contract	Mt. Sinai School of Med. Rosenfield, R.E.	Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or more (over 7-10 mls per 24 hrs) of Human Blood in Human Feces	R-27
New Contract	Roswell Pk. Mem. Inst. Barnard, E. A.	Biochemical Markers or Enzyme Changes that May Presage the Presence of Cancer	R-28
New Contract	Downstate Med. Center Damadian, R.	Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues	R-29
New Contract	Oklahoma U. Health Sci.Ctr. Haberman, J. D.	Proposal for Thermography Technologist Training Program	R-30
New Contract	Rochester Univ. School of Med. & Dentistry Fischer, H. W.	Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy	R-31
New Contract	Stanford Univ. School of Med. Lederberg, J.	Biochemical Markers or Enzyme Changes that may Presage the Presence of Cancer	R-32
New Contract	George Wash. Univ. Davis, D. O.	Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors	R-33
New Contract	Arizona Univ. Medical Ctr. Present, A. J.	Demonstration Project in Earlier Detection of Breast Cancer	R-34
35024	Cincinnati College of Med. Moskowitz, M.	Demonstration Project in Earlier Detection of Breast Cancer	R-35

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45064	Duke Univ. Lester, R. G.	Demonstration Project in Earlier Detection of Breast Cancer	R-36
45058	Albert Einstein Med. Ctr. Isard, H. J.	Demonstration Project in Earlier Detection of Breast Cancer	R-37
35016	Emory Univ. Powell, R. W.	Demonstration Project in Earlier Detection of Breast Cancer	R-38
New Contract	Ellis Fischel State Cancer Hospital Donegan, W. L.	Demonstration Project in Earlier Detection of Breast Cancer	R-39
45062	Georgetown U. Abernathy, M.	Demonstration Project in Earlier Detection of Breast Cancer	R-40
New Contract	Good Samaritan Hospital and Medical Ctr. Goodman, M. J.	Demonstration Project in Earlier Detection of Breast Cancer	R-41
35004	Guttman Breast Institute Strax, P.	Demonstration Project in Earlier Detection of Breast Cancer	R-42
45067	Iowa Lutheran Hospital Young, D. C.	Demonstration Project in Earlier Detection of Breast Cancer	R-43
35026	Kansas Univ. Med. Ctr. Humphrey, L. J.	Demonstration Project in Earlier Detection of Breast Cancer	R-44
35012	Louisville U. Moore, C.	Demonstration Project in Earlier Detection of Breast Cancer	R-45
35028	Virginia Mason Hospital Carlile, T.	Demonstration Project in Earlier Detection of Breast Cancer	R-46
45068	Samuel Merritt Hospital Schweitzer, R.	Demonstration Project in Earlier Detection of Breast Cancer	R-47
45049	Michigan Univ. Whitehouse, W.M.	Demonstration Project in Earlier Detection of Breast Cancer	R-48
35026	Mountain States Tumor Institute Popma, A. M.	Demonstration Project in Earlier Detection of Breast Cancer	R-49

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35006	New Jersey College of Med. & Dentistry Rush, B.	Demonstration Project in Earlier Detection of Breast Cancer	R-50
35020	Oklahoma Univ. Haberman, J. D.	Demonstration Project in Earlier Detection of Breast Cancer	R-51
45046	Pacific Health Research Inst. Gilbert, F. I.	Demonstration Project in Earlier Detection of Breast Cancer	R-52
45065	Pittsburgh Univ. Fisher, B.	Demonstration Project in Earlier Detection of Breast Cancer	R-53
New Contract	Rhode Island Hosp. Constantine, H. P.	Demonstration Project in Earlier Detection of Breast Cancer	R-54
New Contract	St. Joseph's Hosp. Moore, D. L.	Demonstration Project in Earlier Detection of Breast Cancer	R-55
35008	St. Vincent's Med. Center McClow, M. V.	Demonstration Project in Earlier Detection of Breast Cancer	R-56
New Contract	Southern California University, L. A. Guiss, L. W.	Demonstration Project in Earlier Detection of Breast Cancer	R-57
New Contract	Vanderbilt Univ. Hospital Amberg, J. R.	Demonstration Project in Earlier Detection of Breast Cancer	R-58
45047	Wilmington Med.Ctr. Whitney, L. W.	Demonstration Project in Earlier Detection of Breast Cancer	R-59
35018	Wisconsin Medical College Youker, J. E.	Demonstration Project in Earlier Detection of Breast Cancer	R-60
3[illegible]035	University City Science Center Gumnick, J.	Statistical Center for Breast Cancer Demonstration Projects	R-61
33855	New York Univ. Med. Center Lippmann, M.	Study of Intrabronchial Deposition of Inhaled Radioopaque Particles	R-62
45037	Johns Hopkins U. Frost, J. K.	Lung Cancer Control: Detection and Treatment	R-63

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Contract Research Summaries:

Contract No. N01-CB-	Institution Investigator	Title	
New Contract	Alabama Univ. West, S.	Develop and Evaluate New Stains and Other Optical Markers and New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens	S-3
33873	Chicago Univ. Dept. of Obst. and Gynecol. Wied, R. G. L.	Evaluation of Information Requirements for Image Processing of Cell Samples as Basis for Development of Automated Cell Recognition System	S-4
New Contract	Chicago Univ. Bibbo, M.	Studies and Investigations of the Diagnostic Value of Gynecologic Cytopathology Samples	S-5
New Contract	Karolinska Inst. Nobel Med. Inst. Caspersson, T.	Develop and Evaluate New Approaches to the Problem of Markers Applicable to Gynecologic Cytopathology Specimens	S-6
New Contract	Lawrence Livermore Laboratory Biomed. Div. Mayall, B. H.	Development and Evaluation of New Stains and Other Optical Markers Useful for Cytopathologic Specimens in Suspension	S-7
New Contract	Montefiore Hosp. & Med. Center Koss, L. G.	Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens in Suspension	S-8
New Contract	State Univ. of New York Downstate Med. Center Tolles, W. E.	Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathologic Specimens in Suspension and on Slides	S-9

Cytology Automation Contract Program (Cont'd)

New Contract	Papanicolaou Cancer Research Institute Leif, R. C.	Develop and Evaluate New Stains and Other Optical Markers Useful for Clinical Cytopathological Specimens in Suspension	S-10
New Contract	Polysciences, Inc. Halpern, B. D.	Immunological, Enzymatic and Other Biochemical Markers for Detection of Abnormal Cervical Cells on Slides or in Suspensions by Optical Methods	S-11
33862	Rochester Univ. School of Med. and Dentistry Wheeless, L. L.	Investigation of a Slit-Scan Technique as a Basis for an Automated Prescreening System for Cancer Detection in Cytology	S-12
New Contract	Temple Univ. Med. School and Hospital Shulman, J.	Vaginal and Cervical Cell Sample Sources for Cytology Automation	S-13
10055	U.S. Atomic Energy Comm. Los Alamos Scientific Lab. Steinkamp, J. A.	Automated Cancer-Cell Sorting and Analysis	S-14

Cancer Biology Support Program Contract

Contract Research Summaries:

Contract No. NO1-CB-	Institution Investigator	Title	
33863	Baker Histology Laboratory Thomas, L. B.	Maintenance of a Tissue Library and the Processing of Microscopic Slides (NCI 517)	T-1
80047	National Bur. of Standards Computer Serv. D. Ramsay, W. B.	Computer Services	T-2
33887	Pharmacopathics Res. Labs. Tegeris, A. S.	Small Animal Holding Facility	T-3
51072	Smithsonian Inst. Harshbarger, J. C.	A Study of Phylogenetic Aspects of Neoplasia (NCI-538)	T-4

SUMMARY REPORT
OF THE
DIRECTOR
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE

July 1, 1973, Through June 30, 1974

INTRODUCTION

The Division of Cancer Biology and Diagnosis was established in August, 1972, to enhance the research program in the two major directions. In addition to the research efforts in diagnosis and biology, the Division supports a Breast Cancer Task Force Program, which expands to a limited extent into organ site oriented research in epidemiology and treatment. A close coordination of efforts is maintained with the Cancer Control Program in areas of overlapping interest.

The Division's research program may be divided in three broad areas: cancer biology, immunology, and clinical research. The organizational structure roughly follows this same division: first, a group of laboratories performing basic research in cancer biology immediately under the Director; second, two elements, the Immunology Branch and the Laboratory of Cell Biology, working in the immunobiology, immunodiagnosis and immunotherapy of cancer, assigned together with a sizeable contract program to an Associate Director for Immunology; and third, clinical branches working intramurally in the fields of surgery, radiation, dermatology, metabolism, and pathology under an Associate Director for Clinical Research. Clinical research includes both cancer diagnosis and treatment. A fourth organizational element, the office of the Associate Director for Program Planning, performs certain broad administrative functions, but also includes three major contract programs - those in cancer diagnosis, cytology automation, and in breast cancer. There is considerable crossover of research directions between the various research laboratories and clinical branches, and also between major program divisions.

Among the organizational highlights experienced last year, noteworthy is the major expansion of the Cancer Diagnosis Contract Program, concerned both with targeted research and demonstrations of the knowledge gained. Expansion and consolidation also took place in the contract programs in Cytology Automation and Tumor Immunology. The Division was instrumental in setting up a network of 27 Demonstration Projects on Breast Cancer Detection in close cooperation with the American Cancer Society and support from Cancer Control.

CANCER BIOLOGY

Having undergone reorganizational changes, the Laboratory of Biochemistry pursued vigorously research in several directions. In an attempt to define possible structure-function relationship among viral components, high resolution electron microscopy has been used on various proteins selectively extracted from vesicular stomatitis virus. Studies on collagen biosynthesis and secretions are continuing, focusing on the propyl-hydroxylation step, on the quantitation and intracellular localization of the reaction. Another study aims at identification and biochemical characterization of DNA polymerizing enzymes in mammalian cells. One of the isolated enzymes can use natural mRNA as template to form DNA, showing ribonuclease activity, but differing in several respects from the recognized C-type viral reverse transcriptase.

Studies in the area of biochemistry of gene expression involve attempts to determine growth characteristics of cell hybrids after long term culturing, testing the ability of hybrids to cause tumors. Cell hybrids are also used to study control of steroid responsiveness. HTC cells adapted to growth in serum-free medium are studied for nutritional requirements.

Considerable effort is expended on tissue culture work. It has been shown, for example, that fetal antigens may be retained in tissue cultures in excess of five years without changing their expression. A new chemically defined medium for growth of cells in tissue culture has been developed and studies are in progress investigating cell growth under lower than atmospheric O_2 content. Atmospheric O_2 appears to promote karyotypic instability which may be a factor in the evolution of long-term heteroploid cell lines.

Another goal was to elucidate the mechanism by which expression of the histidine operon is regulated in response to the needs of the microorganism under a variety of conditions. Previous work suggested that this system is autogenously regulated -- that is, expression of the genes of the operon is regulated by a protein specified by one of those very genes. The protein in question is known to function as an enzyme, the so-called G enzyme that catalyzes the first step in the pathway for histidine biosynthesis. A role for this enzyme as a regulatory protein that controls expression of the histidine operon is indicated by the findings of several different projects.

Studies in the replication of simian virus 40 DNA showed that it occurs on the nuclear membrane; experiments suggest that mature molecules are released from the membrane upon completion of replication. In another project, investigation is carried out on the DNases present in monkey kidney cells upon SV 40 infection, detecting a variety of DNases by a specially developed high sensitive assay.

Study of the macromolecular processes involved in maturation of bone marrow granulocytes suggests that an enzyme, arginase, plays a role in conversion of myelocytes to mature neutrophil granulocytes. Mature granulocytes contain several times more arginase activity than do immature cells; a 2- to 3-fold increase in arginase activity accompanies the maturation that occurs in short term *in vitro* culture system. The maturation process *in vitro* is greatly enhanced by decreasing the extracellular pool of the amino acid, arginine. The primary and secondary granules of mature leucocytes have been fractionated as confirmed by specific enzymic analysis. These granules are strikingly similar in polypeptide pattern and in glycoprotein composition, despite major differences in their enzyme components.

The main thrust of the Laboratory of Biology is on mammary gland tumors, the transmission of the causative factors and mammary tumorigenesis. The mammary tumor of the mouse with its mammary tumor virus are studied as a model system for the eventual control of breast cancer.

For studying the genetics of the transfer of MTV in crosses between strain C3H-A^{vy}fB with male transmission of an MTV and C57BL presumably without MTV, methods have been developed for the immunofluorescence absorption test for the MTV group specific antigen in the mammary gland. Whole glands appeared negative for the antigen, but a technique for enzymatic dissociation of mammary gland and mammary tumor epithelial cells have now been developed able to detect antigen in the dissociated cells.

Biochemical genetics of the mouse was investigated, working particularly with erythrocyte catalase from BALB/c mice and human beings. The two appear to be indistinguishable by immunochemical methods, but the preparation from humans has a specific activity of 6.8×10^7 mole^{-1} sec^{-1} whereas that from the mice has a specific activity of 2.7×10^7 mole^{-1} sec^{-1}. A twofold difference in RBC catalase between BALB/c and C57BL/6 mice has been shown to be a difference in concentration of catalase in the cell rather than a structural difference in catalase.

Experiments were carried out in DNA methylation in paired normal and neoplastic mouse cell lines _in vitro_, showing that the principal DNA methylation is in 5-methylcytosine. In another study, utilizing an ascites forming ovarian tumor of the mouse, it was shown that the ovarian tumor cells did not enter the lymphatic channels, blocking the drainage of the peritoneal cavity, thus causing the ascites, but that the blockage occurred as a result of layering out of tumor cells over the drainage surface of the peritoneum.

The research in the Laboratory of Molecular Biology continued in the area of cyclic AMP and malignant transformation of cells as well as in the field of genetic investigations.

Transformed embryonic fibroblasts have low levels of cyclic AMP; the decreased adhesiveness, rounded shape, rapid growth and increased agglutinability of transformed cells is due to low levels of cyclic AMP. A mutant of mouse 3T3 cells was isolated in which cyclic AMP levels can be reduced by a fall in temperature. Shortly after cyclic AMP levels fall, the untransformed cells reduce their adhesiveness, retract their processes, round up and become very agglutinable. Prior exposure of the cells to Bt_2cAMP blocks these responses.

Valuable insights into mitogenesis by viruses are coming from studies of the integration of the bacterial virus, lambda, into the E. coli chromosome, and its later excision. When the virus is integrated in unusual sites, it acts as a mitogen, inactivating a variety of different genes. Further geneti damage may occur on virus excision by removal of neighboring bacterial genes. Other work analyzing how lambda decides to lysogenize a cell rather than kill it is directly related to the problem of malignant transformation of cells by viruses. It has been found that regulatory proteins act at a single site on the lambda chromosome, both to activate transcription of gene products favoring lysogeny and to inhibit synthesis of products favoring cell death.

The research effort of the Laboratory of Physiology continued in the direction of investigating the mechanisms of thyroid hormone release, studies of the energy regulation and expenditure and the interdependence of metabolic and behavioral responses. Work has been done in the control of the protein synthesis in the metabolic differences between normal and tumor cells and in molecular and biological effects of radiation.

The objectives of the metabolism studies included how energy expenditure, feeding and general behavior pattern are related to tumor growth, studies of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth, and development of quantitative and reproducible assessment of behavioral output, with hope of detecting early systemic effects of tumors as well as searching for experimental models for cachexia and the decline in food intake observed during neoplastic growth. Work in progress on feeding response to reduced environmental temperature indicates that this response, as well as that to nutrient density, is progressively attenuated with tumor growth. The persistent feeding deficits in animals recovered from lateral hypothalamic damage can be clearly discriminated from cachectic feeding failure.

Quantitative studies of recovery from sublethal irradiation in terms of ability to tolerate a second challenging irradiation show separate patterns for hematopoietic and gastrointestinal systems. Ability to tolerate other agents will also vary according to time after irradiation and system affected. An understanding of the nature and duration of effects of each treatment or combination of treatments is essential in establishing therapeutic programs which can be tolerated by the host.

Studies continue on immunogenetic factors influencing bone marrow transplantation in mice with the goal of avoiding GVH disease so that this procedure can become an adjunct to heavy radiation or chemotherapy in the treatment of cancer.

Radioprotective and radiosensitizing agents are being studied in order t develop new approaches to increase the differential radiation sensitivity of tumors. The protective effects of certain metal ions and of their complexes in reducing the production of free radicals in gamma-irradiated DNA have been further investigated.

The primary objective of the Laboratory of Pathophysiology is the understanding of conditions which determine arrest and regulation of growth in neoplastic tissues. An attempt was made to induce a mammary carcinoma which cou mimic the human counterpart in most of its biological properties. Available mouse tumor models metastasize but are not hormone responsive; rat models are hormone responsive but do not metastasize. Using N-nitrosomethylurea, mammary carcinomas were induced within three months in 90% of Buffalo/N inbred female rats. The tumors are hormone responsive, metastasize consistently and induce hypercalcemia, thus mimicking the human counterparts.

The complexity of the in vivo model system for tumor regression by hormonal deprivation of the host makes difficult a separate analysis of each component of the regression process. An in vitro system in which neoplastic cell populations can be grown in tissue-like structures without interference of host factors, and can be induced to progress, as in vivo, was needed. The development of the capillary culture unit made it possible to grow neoplastic mammary epithelial cells to tissue-like structures on artificial capillaries.

Investigating the role of cyclic AMP in tumor regression, it was established that derivatives of cyclic AMP do inhibit growth of some mammary carcinomas and hepatomas of the rat, this growth inhibition being dose-dependent and reversible upon cessation of treatment. After a single cyclic AMP injection, a sharp increase in activity and quantity of acid RNase is observed exactly as in tumors regressing after hormonal withdrawal. Two cell populations have been isolated from Walker 256 mammary carcinoma and 5123 hepatocarcinoma: one regresses rapidly, the other continues to grow under dibutyryl-cyclic AMP treatment. A simple increase of cyclic AMP level in the tumor is insufficient to produce regression.

An attempt was made to develop cultures of human endothelium to be used for in vitro assay of the tumor angiogenesis factor. Endothelium was obtained from the vein of umbilical cords, treated immediately after delivery with collagenase and trypsin. By an appropriate combination of digestion procedures, it has been possible to obtain pure endothelial cell and capable of accumulating intracellular lipids and undergoing morphological changes similar to those observed in atherosclerotic plaques in vivo.

A group of collaborative projects is being carried on to expand the application of the capillary culture unit to problems outside the area of our immediate concern, but the solutions of which appear both feasible and particularly urgent. The capillary culture unit allows long term culture of a cell population organized in tissue-like structures and under ideal conditions to produce viruses continuously. Attempts are being made to develop an artificial lymphatic system within the culture, so that virus particles can be drained out of the intercellular spaces without disturbing the microenvironment of the culture. Bone marrow dedifferentiates rapidly in standard cultures because special macromolecules required to promote differentiation cannot be preserved after each change of media. An assumption that in capillary culture units these molecules should not diffuse through the capillary wall, so that very small amounts of them present in the pericellular fluid should be sufficient to maintain bone marrow differentiation for long periods proved to be correct, and presently all cell types of the bone marrow can be cultivated.

The activities of the Laboratory of Theoretical Biology fall into several areas: the biophysics of membranes, developmental biology, kinetic-metabolic systems and mathematical and computational methodology and theory related to modeling.

Physical mechanisms for ion transport across the membrane are being investigated, using ionophoric material, isolated from (Na^++K^+) ATPase containing electroplax membranes in a bilayer membrane system. Pore formations have been detected from fluctuating discrete conductance levels, providing new insights into the nature of the membrane pores and their kinetics. The modeling of metabolic systems has reached a high level of complexity and sophistication and on-hand experience in dealing with large systems provides new insights into the theory and methodology of modeling as related to large systems. This activity also serves as a pilot project in establishing model-systems for several important metabolic systems in man.

Work is progressing on the SAAM computer program and supporting activities. New extensions have been added to SAAM and new numerical analysis procedures have been introduced to permit the modeling of more complex systems. A conversational SAAM capability has been implemented on the PDP-10 computer at NIH. The remote terminal software has been improved in reliability and new capabilities have been added for communication with two computers.

IMMUNOLOGY

The rapid development of the field of immunology in recent years has had tremendous application in cancer research. Tumor immunology research comprises the greatest part of the program of three laboratories in the Division of Cancer Biology and Diagnosis. Two of these, the Immunology Branch and the Laboratory of Cell Biology are assigned organizationally under the Associate Director for Immunology. Although the third, the Metabolism Branch, is under the Associate Director for Clinical Research, its program will be summarized in this section. The tumor immunology contract program is reported in the contract section.

The Immunology Branch carries out studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation, applying the information obtained to investigations of the interactions between the immune system and neoplastic cells and to diagnosis and therapy of malignant disease in animal models and in man. The function of cells is partially initiated and modulated by interactions between cell surface receptors and a variety of ligands. One of them, a receptor for antigen-antibody complexes or aggregated IgG, has been partially characterized and appears to play a role in the interaction between non-immune B cells and antibody coated target cells that leads to cell lysis. Human lymphoid cell lines that have cell surface immunoglobulin were shown to have receptors for insulin and growth hormone. T-cell lines, cell lines derived from Burkitt lymphoma and Burkitt lymphoma cells obtained by biopsy appear to lack these receptors. Lymphoid cells from patients with acute leukemia contain a cell surface antigen not found on other lymphoid cells. Antisera have been prepared against this antigen as well as against cells of patients with chronic lymphocytic leukemia and cultured melanoma cell lines. Antisera of the latter group demonstrated an antigen shared in common between melanoma cell lines, explanted melanoma cells and nevus cells. The antigen was not detectable on breast or ovarian cancer cells or skin fibroblasts. Lymphoid cells have been conjugated with a number of haptens, and it has been shown that these modified cells are immunogenic in syngeneic animals as well as in allogeneic animals.

Recent experiments have suggested that some lymphoid cells have a regulatory function relative to other lymphoid cells. Apparent suppressor cell function has been demonstrated by showing that removal of adherent cells from the spleen of tumor bearing mice increased the degree to which non-adherent cells could be sensitized against allogeneic tissue. Adherent cells depress the degree to which non-adherent spleen cells from normal mice can be sensitized against allogeneic tissue. Tumor bearing mice, therefore, seem to produce adherent cells, which can suppress non-specifically cytotoxic immune responses. Adherent cells (B-cells) appear to be better stimulators of MLC interactions, and non-adherent cells (T-cells) appear to be better responders. Separated cell populations have also been studied in allogeneic *in vitro* immunization experiments and have indicated that there is synergy between adherent and non-adherent responders.

In studying the genetic control of immune responses, it is desirable to utilize antigens of known structure. Staph nuclease meets this requirement. H-2 linked genetic control has been demonstrated and localized to the Ir-1 region. Using staph nuclease it was shown that this antigen stimulated protein synthesis in cells of high responder, but not in cells of low responder strains. This assay correlates with cell mediated immunity, thus indicating that genetic control in this system is also exerted over cell mediated immune responses.

In the area of Transplantation Immunobiology, antisera have been prepared which detect a new series of antigens that are controlled by genes located close to the Ir genetic region. They appear to be B-cell differentiation markers.

Patients with various diseases are HL-A typed and associations sought between specific antigens and disease. For example, the normal frequency of HL-A8 is 22%. By contrast, 60% of patients with dermatitis herpetiformis and 90% of patients with gluten sensitive enteropathy carry the HL-A8 antigen. Identification of diseases associated with abnormal frequencies of HL-A antigens may provide clues to the functions of this sytem.

In the Laboratory of Cell Biology, studies of the biologic properties of several solubilized VSTA and TSTA continued. Sensitive assays have been used to characterize the soluble antigen on RBL-5 leukemic cells. The evidence is now clear that the antigen involved (VSTA) in tumor rejection is not infectious virus, budding from the cell surface nor is it viral envelope antigen, but probably a virus-specified new cellular antigen analogous to the VSTA of DNA virus-induced membrane antigens such as the SV-40 antigen on mKSA cells.

Further, a molecular entity with the characteristics of a tumor antigen of the transplantation type has been solubilized from a crude membrane preparation of RBL-5 cells, an RNA virus-transformed neoplasm. A sensitive, simple, and reproducible in vitro technique to monitor the tumor antigen for further purification and characterization is now available.

Studying the mechanisms involved in the immune rejection of syngeneic neoplasms, specifically the SV40 virus transformed tumor mKSA, a search for specific antibody to the tumor was made: no cytotoxic or lymphocyte dependent antibody could be found in immunized or tumor bearing mice; however, low levels of antibody were detected by the isotopic antiglobulin technique.

Investigations are continuing to elucidate at what point in the rejection process lymphocytes from tumor bearing animals are blocked in their expression of cytotoxicity to the tumor. Although unreactive in the Winn assay, tumor bearing lymphocytes proliferate upon exposure to tumor specific transplantation antigens, and are capable of adoptively transferring systemic immunity to irradiated syngeneic recipients.

Early tumor growth was associated with detectable cellular immunity as measured by a number of assays. Animals bearing advanced tumors appeared to have lost immunity detected by direct tumor challenge and the Winn test, whereas specific immunity detected by the microcytotoxicity assay persisted, showing that the immune status of tumor bearing mice is altered with increasing total tumor load.

Cell-mediated immunity has been studied in the MSV system by the lymphocyte stimulation assay. During the period of transient tumor growth, the ability of B cells to respond, even to mitogens, is markedly depressed. When the tumor regresses, reactivity returns to normal. With progressive growing tumors the defect remains marked until the death of the animals. This has been shown to be due to the presence of a suppressor cell in the spleen, which appears to be an activated macrophage.

A series of assays for measuring the general cell-mediated immune competence of cancer patients have been established: enumeration of the percent of T cells in the peripheral blood by rosette formation with sheep erythrocytes, cytotoxic reactivity against human lymphoid cell lines, and lymphocyte stimulation by mitogens, antigens, and mixed lymphocyte cultures. Cryopreserved human lymphocytes retain their functional activities in each of these assays. This resulted in a decrease in the variability of the assays providing a consistent standard.

The work on clinical application of sensitive quantitation of tumor markers found in the serum of individuals with various neoplastic diseases continued. The major emphasis has been with alpha-fetoprotein (αFP) but also has included human chorionic gonadotropin (hCG) and carcino-embryonic antigen (CEA). The usefulness of αFP as a marker for gastric and pancreatic cancer has been demonstrated by a large survey of patients with gastrointestinal cancer in collaboration with the Mayo Clinic. Work has also been done in the area of combining two markers to see what added benefit might be derived. This has been shown to be of vital importance for mixed tumors of testicular origin, but also for gastric and pancreatic cancer. The value of these markers for following patients to gauge the effectiveness of therapy, and especially to predict the earliest time of tumor recurrence after remission, has been demonstrated.

Studies in viral oncology showed that a virus, designated Dl-MuLV, isolated from a mouse bearing RCN-B, showed unique type-specific envelope antigens. By in vitro neutralization tests and immuno-electron microscopy, the viral envelope antigen (VEA) of this virus was shown to be distinct from the VEA of a number of different murine type-C viruses. However, a cross-reacting VEA was found on a subpopulation of AKR-MuLV and a radiation-induced leukemia virus of A strain mice.

The antigen of Dl tumor, a lymphoblastic leukemia induced in SJL/J mice by neonatal inoculation of Dl-MuLV, was also shown to be unique in that none of the virus-coded membrane antigens of the cell, such as GCSA or FMR, or alloantigens PCl and G_{ix} associated with MuLV infection was present on the Dl cell surface.

These findings contributed to the development of an important concept that there exists in various mouse strains a wide variety of type-C viruses antigenically different from each other. This concept is in contrast to the commonly held view that all type-C viruses spontaneously arising in murine tissues and tumors are the same or variants of Gross-MuLV.

The research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, immunological investigations are carried out to determine the nature of the immune processes of importance in the surveillance against neoplastic cells and to define the defects in the immunological system that result in an increased incidence of neoplasia, as well as developing techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy. The second goal is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Several significant achievements have been made in the last year.

Sensitive quantitative radioimmunoassays for oncofetal proteins such as alpha-fetoprotein (AFP) and the beta chain of human chorionic gonadotropin (hCG-β) have been established and evaluated as tests for the diagnosis and for the monitoring of treatment of different forms of cancer. An elevated serum AFP level (> 40 ng/ml) was demonstrated in a significant number of the 700 patients studied. In contrast, only 1 of 500 controls or patients with chronic nonhepatic diseases had a level over 40 ng/ml. In recent studies it was shown that the combined determination of several oncofetal markers can significantly increase the detection rate of cancer.

A significant effort has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific circulatory and cellular immune response to antigens. Antibodies are produced by cells originating in the bone marrow that mature by interaction with a central lymphoid organ to form B lymphocytes, which in turn terminally differentiate into cells synthesizing immunoglobulins. B lymphocytes may be identified because they have immunoglobulins as well as receptors for the third component of complement and for antigen-antibody complexes incorporated into their cell surface membranes. Immunodeficiency diseases in man associated with hypogammaglobulinemia may represent defects in the formation of B cells or defects in the terminal differentiation of immunoglobulin bearing B cells into immunoglobulin producing and secreting cells. Therefore, a technique for the study of the terminal differentiation of B lymphocytes has been developed to define the nature of this critical process and defects in this process in patients with immunodeficiency disorders.

Studies of patients with immunodeficiency disease and a high incidence of malignancy, including patients with the Wiskott-Aldrich syndrome, hypogammaglobulinemia, ataxia telangiectasia and intestinal lymphangiectasia are continuing with the ultimate goal of correcting the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease.

The Wiskott-Aldrich syndrome is an x-linked immunodeficiency disorder with a very high incidence of infections with all classes of microorganisms, and in addition the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that the effector arms of their immune system is largely intact. A most striking feature of the

Wiskott-Aldrich syndrome is the very high incidence of lymphoreticular malignancy found in these patients. Sera from a patient with reticulum cell sarcoma of the brain were studied for antibody to the Papova virus, and about 14 months prior to the clinical appearance of the tumor, the patient developed a striking antibody response.

In other studies the relationship between histocompatability type and gluten-sensitivity disease was further explored. It was found earlier that in dermatitis herpetiformis (DH), a skin disease associated in many instances with gluten-sensitive enteropathy, HL-A8 occurred at a generally increased frequency. In the present studies, it was shown that in two separate populations HL-A8 was especially frequent (80-90% incidence) in those patients with DH who have gastrointestinal disease and infrequent in those patients with DH who do not have gluten-sensitive disease (20%). The implication of this finding is that HL-A8 acts in cooperation with other genes to produce gluten-sensitive disease.

In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase. Specific, sensitive radioisotopic assays for these enzymes have been developed, using labelled substrates and quantitating the products formed by ion exchange chromatography. With these assays the activities of the proline metabolic enzymes in circulating leukocytes in cultured cells undergoing a variety of perturbations are under study.

A method for the measurement of uroporphyrinogen I synthetase in red cells has been developed. The method has been shown to separate heme from the porphyrins and thereby eliminate errors caused by quenching from heme. Because of the high degree of separation of heme from prophyrins, the method is adaptable to various volumes and dilutions of enzyme and can be used in different tissues. The optimal conditions for measurement of the enzyme have been worked out in detail and the method is highly reproducible.

CLINICAL RESEARCH

The area of clinical research includes cancer surgery, radiotherapy, work on skin cancer, and general cancer diagnosis. The branches covered here are Surgery, Radiation, Dermatology, and the Laboratory of Pathology. The program of the Metabolism Branch was reported under Immunology, and the Diagnosis contract program is included with Contract programs. The Surgery Branch carries out active clinical and laboratory research programs, but the staff is also heavily involved in consultative and operative assistance to all of the intramural elements of the National Institutes of Health. The ongoing clinical investigative program of the Branch can be categorized into four areas of chief interest: cancer of the soft tissue pelvic structures, head and neck area, soft tissue and bone sarcomas, and melanomas. There is expanded programming with radiotherapy, chemotherapy (cytotoxic and anticoagulative agents), cryotherapy, and collaborative monitoring through immunological surveillance. The role of radical surgery in the successful management of patients with radiation recurrent cancer of the cervix and vagina has been well established. Based upon new evidence indicating that the presence of an isolated mass of tumor cells which might remain subsequent to conventional head and neck therapy might be highly sensitive to adjuvant therapy regimes, the Methotrexate study has been expanded. Preoperative use of this agent has shown an 81% primary tumor response rate without altering surgical morbidity. New surgical techniques are under development both for tumor removal and for reconstruction of defects. All new sarcoma patients are entering an anticoagulant study. Although there has been some postoperative morbidity, preliminary results suggest there may be some reduction in the propensity to metastases of these tumors.

The Surgery Branch is currently collaborating with the Immunology Branch and the Chemotherapy Branch in a protocol study to assess the effectiveness of immunotherapy or chemotherapy in patients with minimal residual melanoma. The chemotherapy arm consists of administration of methyl CCNU. The immunotherapy arm consists of administration of BCG and allogenic cultured melanoma cells. The possible utilization of "combination immunotherapy" is being investigated based on experiments showing that in four species, including man, agents which promote delayed hypersensitivity reactions increase the responsiveness of the circulating lymphocytes to blastogenesis by phytohemagglutinin. A combination of agents which produce a delayed hypersensitivity reaction seems to be more potent than single agents in causing the enhanced lymphocyte reactivity. It is possible that "combination immunotherapy" may be more potent than "single immunotherapy."

A Breast Oncology Section has been established to do clinical research in all aspects of breast cancer from early detection through primary surgical treatment, to adjuvant chemotherapy programs and treatment of metastatic disease. The Branch is involved also in aspiration biopsy, primary surgical treatment, endocrine ablative procedures, and staging for all the patients entered into the service. The program is being rapidly expanded and developed along the lines of already prepared randomized protocols to be of maximum benefit to the patients. In collaboration with the Laboratory of Pathology, fine needle aspiration biopsy for the cytologic diagnosis of solid tumors is being evaluated. Results indicate that diagnoses of cancer obtained on the basis of fine needle aspiration have been confirmed in all cases in subsequent histologic examination of excised tissue. A negative report, however, does not rule out malignancy.

To determine the usefulness of in vitro lymphocyte reactivity to phytohemagglutinin (PHA) and dinitrochlorobenzene (DNCB) contact sensitivity combined as determinants of the tumor status of cancer patients, cellular immune competence was determined in postoperative patients considered clinically free of tumor and compared with preoperative patients and normal controls. The immune reactivity of the cured cancer patients correlated with the histology of the tumor previously excised. Patients cured of squamous carcinomas had high incidence of impaired lymphocyte reactivity and DNCB contact sensitivity similar to those found in preoperative patients. Patients with sarcomas, melanomas, and adenocarcinomas had lymphocyte reactivity higher than their preoperative counterparts and normal subjects, and did not display the abnormalities of DNCB contact sensitivity present in comparable preoperative groups. Among patients cured of cancer, as in preoperative patients, only those with squamous carcinomas and sarcomas had serum suppressants of lymphocyte reactivity. The persisting high incidence of impaired cellular immunity in patients cured of squamous carcinomas indicates the need for investigation of genetic and environmental factors which may be responsible for the defects and the determination of their relation to tumor induction. The intact cellular immune competence of patients clinically cured of sarcomas, melanomas, and adenocarcinomas indicates that patients treated for tumors of these histologic types who display abnormalities by the assays used may be suspect of having residual tumor.

The in vitro spontaneous lymphocyte rosette (T cell) assay was used to determine cellular immunologic competence in patients with bronchogenic carcinoma. Among preoperative patients with localized tumors, T cell levels were significantly lower than in normal controls. With advanced stages of disease, T cell levels declined progressively among patients with squamous cell carcinoma, oat cell carcinoma, and undifferentiated carcinoma, but not among patients with adenocarcinoma. Squamous carcinoma patients considered cured had persisting low T cell levels, but cured adenocarcinoma patients had normal levels. The data indicate that T cell levels correlate with prognosis and clinical course of patients with bronchogenic carcinoma. The assay may, therefore, provide a rational basis for the selection of patients who are at high risk for the development of metastases after surgical resection and who may benefit from the early institution of adjunctive therapy.

Results of another study suggest that monitoring peripheral blood T cell levels may provide criteria for predicting response to radiation therapy and prognosis after treatment, and may be useful for the earlier selection of patients who may benefit from adjuvants such as chemotherapy or immunotherapy.

A pilot study is underway to evaluate the affinity of isotopically labelled Bleomycin for primary and metastatic neoplasms via total body rectilinear scanning and regional gammagraphic examination. Through its interaction with DNA, Bleomycin might aid in localizing clinically unsuspected metastatic disease.

Results of laboratory research include a demonstration in mice that BCG vaccination reduces the incidence of melanomas after inoculation of tumor cells. In guinea pigs, surgical interference with afferent lymphatic drainage, or removal of lymph nodes, did not prevent rejection of tumor allograft; systemic sensitization of the host eventually occurred.

The program of the Radiation Branch is devoted entirely to studies in patients. The staff of the Radiation Branch has maintained a high level of collaboration with other program areas and has continued to provide considerable advisory support to both intramural and extramural areas. Patient consultations, irradiation exposures of biological materials and animals represented the bulk of cooperative effort.

The program on Ewing's sarcoma has received national attention, having markedly increased the survival for patients with advanced metastatic disease and given a 5-year survival rate of 50% for the more localized form of this disease. The presence of an active immunologic response has been documented in some patients and cultures of Ewing's sarcoma cells have now been established *in vitro* as a preliminary step to development of a tumor vaccine for diagnostic studies and potential immunotherapy.

Long-term observation of patients treated for Hodgkin's disease between 1965 and 1969 has continued to yield important data with respect to the diagnostic methods of staging, efficacy of the therapeutic techniques employed, and the implications of the basic disease as well as its treatment for both survival and the quality of life. The 9-year survival rate for patients with "early" stage Hodgkin's disease is 90%.

The Dermatology Branch conducts both clinical and basic research on the etiology, diagnosis, and treatment of malignant diseases involving the skin and the host's response to these diseases. In patients with *Xeroderma pigmentosum*, rare autosomal recessive dermatologic disease, characterized by pigmentary changes, telangectasia, atrophy, and tumor formation in response to even minimal sun exposure, a defect (presumably in an endonuclease) in repair of UV damage to DNA has been found in XP cells. Cells from several tissues have been obtained from these patients and shown that other cells than the normally used skin fibroblasts are suitable for studying the DNA repair defect in XP. Peripheral blood lymphocytes rather than dividing cells largely overcome the problem of the repair synthesis being obscured by semiconservative DNA synthesis. A form of XP was discovered in which the patient's cells had no demonstrable DNA repair defect, despite the presence of severe skin disease, including numerous malignant tumors. Cells from this patient and other XP patients are being studied to determine the role of DNA repair in tumor formation after exposure to ultraviolet radiation of chemical carcinogens which react with and distort DNA.

Malignant melanoma is a highly malignant tumor in which production of melanin is often increased. The melanin granules in tumors are morphologically distinguishable from those found in normal cells. Melanogenesis and its regulation in normal and malignant cells is being investigated to determine the relationship of this process to melanoma formation; cells from various pigmentary mutants of the mouse have been compared to elucidate the genetic basis for control of melanin production. Melanogenesis followed a common pathway of melanosome formation; the major difference noted among the mutants was the premature termination of melanization of the granules at different stages of melanosome formation. Melanosomes from black mice contain seven proteins, six of which are of extremely high molecular weight. Melanogenesis in the mouse is dependent upon tyrosinase activity rather than peroxidase activity. Tyrosinase activity is under allosteric control and phospholipids play an important role in the expression of the enzyme's activity. Tyrosinase activity in black mice seems to be under the control of a heat-labile, low molecular weight inhibitor. Tyrosinase activity in albino mice can be demonstrated when tissue homogenates are pretreated with detergents. Finally, it has been found that some proteins of malignant melanoma melanosomes are unique and not found in normal melanin granules. These aberrant proteins found in malignant tissue could be used in developing an immunotherapeutic approach against melanomas.

Interesting results come from the study of Sezary syndrome - a lymphomatous disease characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood. Ultrastructural studies have shown the large circulating cells to have a characteristic convoluted nucleus. In addition, the Sezary cell has been shown to be lymphocytic, to have chromosomes abnormal in both number and structure. Using quantitative measurements of nuclear contour index and cell diameter, it is possible to quantitate the nuclear irregularity of the Sezary cell in comparison to other abnormal leukemic cells. It has been found that a cell with a nuclear contour index above 8.6 in the circulating blood is a Sezary cell.

Other studies include investigation of cell-mediated immunity and contact sensitization in human skin, demonstrating a temporal relationship between _in vivo_ reactivity _and in vitro_ specific lymphocyte transformation during primary immune response. Preliminary procedures have been worked out for the isolation of tonofilaments from bovine epidermis. Finally, a new procedure employing both scanning electron microscopy and light microscopy has been developed in order to visualize non-attached cells fixed in suspension and recovered in high yield.

Although the Laboratory of Pathology is heavily involved in the training of residents in anatomic pathology, and in histopathology and cytopathology services for patients throughout the Clinical Center, it also carries out a broad research program with major emphasis in viral oncology and other aspects of carcinogenesis. Noteworthy research results include:

Demonstration of the B cell origin of human nodular lymphomas of the poorly differentiated lymphocytic and mixed cell types, using methods for demonstrating cell surface markers for B cells and monocyte-macrophage markers on the neoplastic cells of histiocytic medullary reticulosis;

Evidence that in asbestos carcinogenesis, fiber size is a critical factor;

Identification of the initiation of viral protein synthesis as the site of the anti-viral action of interferon;

Ultrastructural demonstration of herpes virus antigens on cell surfaces with hybrid antibodies;

Characterization of immune responses in animals infected with the oncogenic monkey herpes virus, H. saimiri, and finding that antibodies to early antigens of the virus are related to development of tumors;

Transformation of mouse salivary epithelium by polyoma virus in transfilter interaction;

Conclusion that histopathologically mycosis fungoides is a distinctive entity and that its pattern of spread in lymph nodes and spleen mimics the normal migration of T-lymphocytes.

Considerable progress has been made in the area of the automation of cytology. Since most of the work has been done under a contract supported program, it is reported in the contract section.

CONTRACTS PROGRAM

The Division of Cancer Biology and Diagnosis supports four programs of contract research. The greatest effort is in the area of Tumor Immunology, managed by the office of the Associate Director of Immunology. The program is divided in three parts: immunobiology, immunodiagnosis and immunotherapy. Immunobiology concentrates on fundamental problems like identification and characterization of surface membrane components of tumor and immune system cells, immunologic reactivity of hosts to tumors and identification of immune cells involved. Evaluation of the diagnostic feasibility of CEA test, cell-mediated immune response, and of humoral immune response to tumor associated antigens or various serologic assays is the province of the immunodiagnosis program. The work in immunotherapy is mostly preclinical, although several human studies have been already initiated.

The contract program of the Breast Cancer Task Force is the oldest one in this Division, directed toward multidiscipline approach to this very common cancer. It is comprised of four subdivisions: diagnosis, epidemiology, experimental biology and treatment, and its management, is the responsibility of the Breast Cancer Program Coordinating Branch. The diagnosis program is further subdivided into detection (development, refinement and evaluation of instrumentation); immunology (development of long term tissue cultures of mammary epithelial cells, and development of immunologic assays for breast cancer associated antigens); and diagnosis-prognosis segment investigating the hormonal markers. Epidemiology supports some programs of viral etiology and hormonal mechanisms, but its principle stress is on studies in the genetics of breast cancer. The biggest program is that of experimental biology, directing its efforts to in vitro cultivation of mammary cells, attempting to assess the biological characteristics of mammary epithelial cells, studying the pathophysiology of mammary tumors and their hormonal responsiveness. Finally, the growth of a mammary tumor, its metastasizing capacity and regression are being investigated. The treatment program emphasized three main areas: evaluation of the relative effectiveness of current surgical methods, search for new methods of measuring cancer cell growth potential and evaluation of various markers for monitoring therapy.

The contract program of the Diagnosis Branch experienced a rapid expansion last year. Most of the effort was devoted, in cooperation with the American Cancer Society, to setting up demonstration projects for earlier detection of breast cancer, funded through the Division of Cancer Control. Three lung cancer projects have been negotiated, aimed at screening heavy smokers. Considerable effort has been spent for improvement of X-ray imaging, evaluation of the computer assisted tomography of the brain, study of nuclear magnetic resonance in application to normal and neoplastic tissue, and development of proton beam for densitometry of soft tissue. Other projects of interest covered establishment of a registry of patients who underwent intensive chemotherapy or radiation therapy for cancer, investigation of biochemical and hormonal markers for early detection of cancer, development of a test for human blood in stool specimens, preparation of bowel prior to colonoscopy, and finally development of methods for earlier diagnosis of pancreatic cancer.

Another rapidly expanding contract program area included in the Diagnosis Branch was the automation of cytology, emphasizing development of instruments for sensing-screening and decision-diagnostic systems. A major commitment was made to investigations of preparation and stoichiometric staining of clinical specimens and quantification of the numbers and types of cells present on cytologic smears.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE.
SUMMARY STATEMENT
July 1, 1973 through June 30, 1974

During the past year, the Laboratory of Biochemistry has undergone major organizational changes. They may be summarized as follows: 1) The Nucleic Acid Section (Dr. W. Schneider, Head) was moved out of the Laboratory; 2) the Cytochemistry Section (Dr. D. Burk, Head) was dissolved; 3) a new Chief (Dr. R. Goldberger) was appointed; and 4) four new Sections were added -- Nucleic Acid Enzymology (Dr. M. Singer, Head), Cellular Physiology and Oncogenesis (Dr. K. Sanford, Head), Biochemistry of Gene Regulation (Dr. E. Thompson, Head), and Cellular Regulation (Dr. R. Goldberger, Head). A sense of excitement is in the air as the Laboratory begins this new phase in its long and varied history.

The work of the BIOSYNTHESIS SECTION (Dr. E. Kuff, Head) has been carried out by four independent groups:

1. Dr. E. Shelton and her colleagues have focussed their efforts on two projects. One involves very high resolution electron microscopy of defined macromolecules. Besides studying two purified bacterial enzymes, they are currently examining preparations of vesicular stomatitis virus from which various proteins have been selectively extracted. This work (in collaboration with R. A. Lazzarini, NINDS) was undertaken to define possible structure-function relationships among the viral components. The second project represents a long-term effort to detect and characterize types of cell-cell interactions that occur during development of the immune response. Short-term cultures of mouse peritoneal cells have been studied by correlated light and scanning electron microscopy. Evidence for cell recognition and interaction is provided by characteristic cell-clustering patterns and by the occurrence of highly oriented cell surface projections between neighboring cells.
2. Dr. B. Peterkofsky and her coworkers are continuing studies on aspects of collagen biosynthesis and secretion. Previous work showed that collagen synthesis takes place on ribosomes bound to the endoplasmic reticulum. Attention is now focussed on the prolyl-hydroxylation step, particularly the quantitation and detailed intracellular localization of the reaction. Ascorbate stimulation of collagen secretion in normal cells was correlated with its effect in increasing hydroxylation of proline and lysine. In contrast to "normal" 3T3 cells, a virus-transformed line, Ki-373, showed no dependence on ascorbate for maximal proline hydroxylation or for collagen secretion. Dibutyryl cyclic-AMP had no effect on collagen synthesis in 3T3 cells but caused increased synthesis in transformed cells at dose levels in the range of those that influence cell morphology and growth rate.
3. Dr. S. Wilson and his colleagues have engaged in two major projects. One involves a program on the identification and biochemical characterization of DNA polymerizing enzymes in mammalian cells. At least 5 activities, probably representing distinct enzyme forms, have been separated from mouse myeloma. One of the enzymes, which appears to be associated with the cytoplasmic membranes, is of particular interest since it can use natural mRNA as template to form DNA and shows ribonuclease H activity but differs in several respects from the recognized C-type viral reverse transcriptase. An apparently identical enzyme has been detected in normal adult liver microsomes. The second project deals with a collaborative study (J. Minna and T. Marshall,

NHLI; K. Stromberg and A. Gazdar, NCI) on oncornovirus expression in human x mouse hybrid cells, partly in an effort to detect human tumor virus expression. Several hybrid lines that segregate mouse chromosomes have been found to spontaneously express virus; however, no evidence has yet been obtained that this is not murine leukemia virus.

4. Dr. E. Kuff and coworkers are continuing studies on mouse tumor-associated intracisternal A-type particles. Synthesis of A-particle protein has been studied in several particle-rich neuroblastoma cell lines. The major structural protein is more rapidly labelled and turns over more rapidly than the average cell protein. Since no evidence could be found for transfer of particles to the culture medium, it is likely that their turnover is an intracellular process. A possible consequence is the reentry of particle-associated components, such as DNA polymerase and nucleic acids, into the metabolic compartment of the cells. RNA extracted from purified myeloma A-particles has also been examined. About 12% of this RNA has properties of size and polyA content consistant with mRNA. High molecular weight (60-70S) RNA was not found.

Four major projects are currently underway in the BIOCHEMISTRY OF GENE EXPRESSION SECTION (Dr. E. Thompson, Head):

1. Growth control in cell hybrids. Neither L cells nor HTC cells show density-dependent growth; their hybrids often do. Studies are in progress to determine whether parental characteristics reappear in these hybrids after long-term culture, to follow the stability and chromosome content of these hybrids, and to test the ability of hybrids to cause tumors.

2. Other aspects of gene expression in hybrids. Cell hybrids are being used to study control of steroid responsiveness and regulation of expression of aryl hydrocarbon hydroxylase. One example is to determine whether hybridizing a steroid receptorless L cell with a receptor-containing L cell will permit steroid response. Cell enucleation methods are also being developed to study separately nuclear and cytoplasmic effects in hybrids.

3. Steroid receptors are being examined in human breast cancer tissue to determine whether they can be of predictive value in choice of therapy. Similar studies in certain human leukemias are also planned.

4. HTC cells adapted to growth in serum-free medium are being studied for nutritional requirements. These cells have been found to produce a rat serum protein. Synthesis of this protein is under investigation.

The efforts of the CELL PHYSIOLOGY AND ONCOGENESIS SECTION (Dr. K. Mifflin, Head) involve five major projects:

1. Rapid detection of cancer cells _in vitro_. A combination of cytologic criteria have been used to diagnose spontaneous and chemical carcinogen-induced neoplastic transformation of rat, hamster, and mouse cells _in vitro_. Although no single morphologic change was of predictive value, combinations of morphologic features showed a high correlation with tumor production _in vivo_ and cytodiagnosis of 24- to 48-hour coverslip preparations appears to be generally feasible.

Four other diagnostic methods are currently being evaluated and correlated with results of _in vivo_ assay. These are colony morphology in quantitated plated cell preparations, plating efficiencies in agar, plating efficiencies in low serum (1% as compared with 10%), and susceptibility of cells to activated macrophages.

2. Studies on nucleic acid methylation. Several studies relevant to the "aberrant methylation" hypothesis of malignant transformation were undertaken. The C-type sarcoma viruses were found to have two methylase activities (N^2-guanine and 1-methyladenine) whereas the leukemogenic viruses have only one (N^2-methylguanine). In contrast to the C-type RNA tumor virions, the N^2-guanine methylase activity of the intracytoplasmic A-particle associated with the mammary tumor virus remains with the particle after surfactant treatment. Extracts of normal lactating mammary tissue of the mouse were found to have greater methylating capacity than extracts of any mouse mammary tumor tissue. This is the first noted exception to the dogma that hypermethylation is associated with malignant conversion.

A DNA methylase activity (5-methylcytosine) was found to be associated with a nuclear membrane fraction that contains DNA polymerase activity; also, a new activity, 1-guanine methyltransferase, was discovered. These observations suggest that nucleic acid fractions other than tRNA may also be involved in aberrant methylation.

3. Physiologic studies on neoplastic transformation *in vitro*. The frequency of neoplastic transformation within single cell clones has been found to be higher in medium supplemented with horse serum than in medium supplemented with bovine serum. This observation is important in eliminating the selection of differentiated cell types as the mechanism of the serum effect on malignant change. The study has also provided paired neoplastic and non-neoplastic cell lines, both derived from the same cell, for comparative studies.

A study on fetal antigens in tissue cultures showed that these antigens can be retained even after 5 years in long-term lines derived from fetal tissues and that no consistent qualitative or quantitative changes in their expression were associated with spontaneous neoplastic change *in vitro*. The cell lines used in this study might be useful for immunodiagnosis or immunotherapy since they share some fetal antigens with various tumor cells.

4. Development of a chemically defined medium for growth of cells in tissue culture. A study was completed on the new chemically defined medium, NCTC 163, which, in the absence of serum or proteins, gives a higher yield of cells from long-term lines of diverse species than does medium NCTC 135. When supplemented with autoclaved peptone, this medium was found to facilitate the establishment of cell lines from freshly explanted mouse fetal tissue. An autoclavable peptone dialysate was found to facilitate the adaptation of mammalian cells to growth in chemically defined medium. Attempts to fractionate this dialysate have not yet yielded information on what component(s) is responsible for its effect.

5. Effect of oxygen tension on cells in tissue culture. In current plating methods cells are grown under atmospheric O_2. Equal or better plating efficiencies were obtained in cultures of rodent fetal cells, either normal or malignant, when grown under a gas phase containing 1% O_2. The reducing agents, cysteine and thioglycollate, did not alleviate the growth inhibition observed at 20% O_2. The growth inhibition was also independent of type of medium, source of serum, or use of a feeder layer. Further studies on fetal human and adult monkey cell cultures suggest that enhanced colony development in low O_2 is a general phenomenon applicable to all cultured cells, regardless of species. Freshly explanted or normal diploid cells appeared more sensitive to 20% O_2 than did long-term heteroploid cultures of fetal or adult origin. A separate

study indicates that atmospheric O_2 promotes karyotypic instability and thus may be a factor in the evolution of long-term heteroploid cell lines.

The principle goal of the CELLULAR REGULATION SECION (Dr. R. Goldberger, Head) has been to elucidate the mechanism by which expression of the histidine operon is regulated in response to the needs of the microorganism under a variety of conditions. Previous work suggested that this system is autogenously regulated -- that is, expression of the genes of the operon is regulated by a protein specified by one of those very genes. The protein in question is known to also function as an enzyme, the so-called _G_ enzyme that catalyzes the first step in the pathway for histidine biosynthesis. A role for this enzyme as a regulatory protein that controls expression of the histidine operon is indicated by the findings of several different projects:

1. Strains have been isolated in which a single mutation in the structural gene for the _G_ enzyme leads not only to synthesis of structurally damaged _G_ enzyme but also to loss of regulation of the entire histidine operon.
2. The _G_ enzyme has been shown to interact with high affinity and high specificity with aminoacylated histidyl-tRNA. Such properties fulfill an important requirement for any regulatory protein that may influence expression of the histidine operon. The enzyme does not, however, differentiate between normal (wild type) histidyl-tRNA and immature (_hisT_) histidyl-tRNA. This finding eliminates the possibility that the difference in the regulatory properties in the two species of tRNA is mediated by _G_ enzyme recognition and suggests that the difference is mediated by the interaction of a _G_ enzyme-tRNA complex with the DNA of the histidine operon.
3. The _G_ enzyme has been found to interact specifically with the DNA of the histidine operon carried in the genome of a defective ϕ80 transducing bacteriophage. Furthermore, competition experiments, using various preparations of radioactively labeled and unlabeled DNA suggest that the enzyme interacts with the regulatory region of the operon.
4. A purified _in vitro_ system was developed for transcribing the histidine operon carried in the genome of a defective ϕ80 transducing bacteriophage. Messenger-RNA specific for the histidine operon was found to be synthesized in this system from the correct ("sense") strand of the phage DNA. This mRNA had a sedimentation constant identical with that of histidine operon mRNA synthesized _in vivo_. Addition of purified _G_ enzyme to the _in vitro_ system caused a severe and specific inhibition of transcription of the histidine operon, demonstrating that the enzyme functions as a negative control element for the histidine operon _in vitro_ and suggesting that it plays a similar role _in vivo_.
5. Studies are under way to develop an _in vitro_ system for synthesizing the enzymes of the histidine operon from the DNA of a defective ϕ80 transducing phage that carries the operon in its genome. Because the _in vitro_ system involves both _E. coli_ and _S. typhimurium_, it is complicated by the problem of the bacterial restriction-modification systems. For this reason, a good deal of effort has gone into obtaining "restrictionless" strains and into other possible ways to solve the restriction problem.

The major concern of the CYTOCHEMISTRY SECTION (Dr. D. Burk, Head) has been the effect of amygdalin on Lewis lung tumors in mice. Large-scale testing in experimental animals has resulted in the accumulation of a large amount of data, interpretation of which has given rise to some controversy.

The aim of one group of the NUCLEIC ACID ENZYMOLOGY SECTION (Dr. M. Singer, Head) is to study, from an enzymological point of view, the replication of simian virus 40 DNA. The approach has been four-fold:

1. Good evidence has been obtained indicating that replication of SV40 DNA occurs on the nuclear membrane. The experiments suggest that mature molecules are released from the membrane upon completion of replication. The primary tool for this work has been the so-called "M-band" procedure for isolation of nuclear membranes from monkey kidney cells. Apart from the relevance of these findings to viral multiplication, they are of more general significance to ongoing investigations on the site of mammalian DNA replication and suggest that at least a portion of that process may occur on nuclear membranes. An investigation of the possibility that isolated nuclear membrane will permit the *in vitro* synthesis of SV40 DNA is now under way.

2. Pradman Qasba, prior to joining the section, had already developed one type of *in vitro* system for SV40 DNA synthesis. He demonstrated that intact nuclei from infected cells carry out SV40 DNA synthesis. Although the nuclei make DNA chains consisting of SV40 sequences, they cannot complete the molecules; therefore, chains less than a genome in size accumulate. The role of RNA as a primer for DNA synthesis is also being studied with these isolated nuclei.

3. Another project has involved investigation of the DNases present in monkey kidney cells upon SV40 infection. Nuclear extracts were prepared and fractionated in DEAE-cellulose columns. A highly sensitive assay, that measures even a single nick in the double-helical circular SV40 genome and that is rapid and simple, was developed. Using this assay, as well as analytical studies in the ultracentrifuge, a variety of DNases were detected. The enzymes were identical in extracts of infected and uninfected cells (BSC-1 line). One of the enzymes appears to be identical with an enzyme reported to be present in mature virions; another is associated (on the column) with a material that binds to the DNA and markedly increases its sedimentation rate. The nature of this "binding protein" and its possible relation to histones is being investigated.

4. Studies are continuing with defective SV40 arising upon serial passage of virus at high multiplicity of infection. The DNA of such virus is shorter than normal SV40 DNA and also contains host sequences in place of certain viral sequences. Preparations of defective virus inhibit the replication of the DNA of "good" virus in monkey kidney cells. The inhibition appears to be competitive. This finding is based on the earlier demonstration that the rate of viral DNA synthesis, at a given time after infection, is dependent on the multiplicity of infection. It is hoped that investigation of the replication of these particles will lead to a study of the recombinational events that occur upon SV40 replication, thus yielding the host-substituted particles.

Another group of this Section has been studying the enzyme, histidine ammonia lyase. The binding of 3-4 cysteines per mole of enzyme (tetramer) is accompanied by a stereo-specific and active site-specific modification and inactivation. The modified structure does not include the cysteine and is marked by a new absorption peak at 340 mμ. A peptide containing the modified group is being analyzed. Further progress has been made on the mechanism of the β elimination of NH_3 from histidine, as catalyzed by histidine ammonia lyase. The rate-limiting step clearly precedes the dissociation of NH_3 from the enzyme: the C-H bond breakage is itself at least partially rate-limiting for

[β-$^{2}H_{2}$]-DL-histidine and for the analog, [β-$^{2}H_{2}$]-4-fluoro-DL-histidine. The rate-limiting step for [β-$^{2}H_{2}$]-4-NO_{2}DL-histidine appears to be different.

Four separate but interacting groups have carried out the investigations of the PROTEIN CHEMISTRY SECTION (Dr. E. Peterson, Head):

1. Dr. Evans and his colleagues, continuing their studies of the macromolecular processes involved in maturation of bone marrow granulocytes, have made observations that suggest that the enzyme, arginase, plays a role in conversion of myelocytes to mature neutrophil granulocytes. They have found that mature granulocytes contain several times more arginase activity than do immature cells and have shown that a 2- to 3-fold increase in arginase activity accompanies the maturation that occurs in their short-term *in vitro* culture system. Moreover, the maturation process *in vitro* is greatly enhanced by decreasing the extracellular pool of the amino acid, arginine. Isolation of arginase from granulocytes has been undertaken for the purpose of preparing a specific antibody. Dr. Evans' group has fractionated the primary and secondary granules of mature leucocytes, as confirmed by specific enzymic analysis, and has compared them by gel electrophoresis in sodium dodecyl sulfate. The results indicate that these granules are strikingly similar in polypeptide pattern and in glycoprotein composition, despite major differences in their enzyme components.

Dr. Evans has been able to obtain human bone marrow cell populations highly enriched with respect to immature granulocytes. He has used this method (in collaboration with Drs. Chabner and Coleman of the Laboratory of Chemical Pharmacology, DCI, NCI) in studies that show that cytidine deaminase activity is markedly lower in immature human bone marrow cells than in mature ones, whether the cells are from a normal individual or from a patient with chronic myelogenous leukemia. These observations support the conclusion that the lower deaminase activity reported for leukemic cells reflects the level of cellular maturation rather than a specific enzymic defect of leukemic cells.

Using fluorescent antibody and cell separation techniques, Drs. Mage and Evans have demonstrated that mature granulocytes from guinea pig bone marrow possess surface antigens that are absent on immature cells. These antigens have also been shown to develop during maturation in the *in vitro* system.

2. Dr. Mage and his colleagues have found that the T-type lymphocytes from spleen that initiate graft-vs.-host (GVH) reactions appear to differ, with respect to antigen binding, from those that mediate cell-to-cell cytotoxic reactions, as indicated by the fact that the latter are removed by absorption to allogeneic target-cell monolayers whereas the former are not. These observations have been confirmed by similar results obtained when spleen cells from the other parental strain were incubated with monolayers of the same F_1 hybrid cells. They are continuing to search for conditions under which the GVH lymphocytes can be bound to target cells.

3. Dr. McBride and coworkers, pursuing their studies on the transfer of genetic information by incubation of recipient cells with chromosomes isolated from other cell strains, have found it possible to assess simultaneously the transfer of two different genes (one sex-linked, the other autosomal) by the use of a suitable selective medium. Thus, they have isolated several clones of transformed mouse L_{A9} cells that contain either active adenyl phosphoribosyltransferase (APRT) or active hypoxanthine-quanine phosphoribosyltransferase (HGPRT), but no clones that contain both enzymes. Human, as well as Chinese

hamster, chromosomes were used in these experiments. Extracts from HGPRT transformants are being examined for the presence of several other X-linked chromosomal donor genes.

Serial No. NCI-5210
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Control of gene expression and growth in animal cells

Previous Serial No.: Same

Principal Investigator: E. Brad Thompson

Other Investigators: L. Lyons, T. V. Gopalakrishnan, A. Venetianer and D. Marciani

Cooperating Units: D. Tormey, MODCT, NCI; M. Meltzer, Biology Branch, NCI; G. Reeck, Lab. of Nutrition and Endocrinology, NIAMDD; S. S. Yang, LCB, NCI; R. Schwartz, Immunology Branch, NCI; Marc E. Lippman and S. Perry, Chemotherapy Branch, NCI; D. Nebert, W. Benedict, and I. Owens, LBS, NICH; J. Cheder and B. Wiggert, NICHD.

Man Years:
Total: 10
Professional: 6
Other: 4

Project Description:

Objectives: Understanding of the mechanism of gene regulation in eukaryotes, particularly as related to steroid hormone action and growth control. Application, where possible, of findings in these areas to clinical situations.

Methods Employed: These have been listed in previous reports. New techniques under development are hybridization of enucleated cells and nuclei; and heterochromatic staining of chromosomes. Use of nuclear binding assays for qualitative assessment of steroid receptors has been added to the techniques employed.

Major Findings:
1) Growth Control Using Cell Hybrids. New HTC x L cell hybrid clones have been shown to display density dependent growth control on the basis of total cells per unit surface area, thymidine labelling, and lack of growth in soft agar (Lyons). In these parameters they differ from either parent. We have observed that hybrids which return to the uncontrolled growth characteristic of the parents invariably prove to be those which have segregated about one quarter of their original 1S + 1S number of chromosomes. We are attempting experiments to show whether this is a causal relationship. We have initiated

studies (Reeck, Marciani) on nuclear acidic proteins, plasma membrane proteins, and concanavilin A binding sites in these hybrids. The hybrids are under test for tumorigenicity in the thymusless, inbred "nude" mouse. They are also being tested for reactivity with activated macrophages, cells which appear to selectively destroy tumor cells (Meltzer).

2) Other Studies of Gene Expression Using Cell Hybrids

a) Hybrids between a subline of 3T3 cells which were benzo [a] pyrene resistant and lacked inducible aryl hydrocarbon hydroxylase (AHH), and HTC cells failed to show the enhanced AHH induction we had observed in HTC x 3T3 wild type cells. This suggests the view that the effect was on the 3T3 enzyme and was not "unmasking" of the HTC enzyme (Nebert, Owens, Benedict).

b) In the HTC x L cell hybrids used for studies of growth control, we also extensively studied steroid effects (Lippman). These hybrids have lost HTC-specific responses to glucocorticoids but retain L-specific responses. Studies of steroid receptors in these hybrids show them to be plentiful, and nuclear binding studies suggest that L cell and HTC cell receptors are distinctive but both present in the hybrid. A steroid-resistant L cell line was developed, shown to lack most steroid receptor, and hybridized with HTC cells. Again the HTC-specific steroid response was lost, but no L-specific responses were seen either. However, steroid receptors (presumably supplied by the HTC cells) were present. Thus HTC receptors could not functionally substitute for L cell receptors.

These data suggest for the first time that not all glucocorticoid receptors are alike and that different specific responses may require distinct cytoplasmic receptors.

c) New hybrids are being made (Venetianer). These include crosses between the above steroid-resistant L cells and will-type L cells, between human diploid lines and HTC cells to test the thesis that the human X carries a repressor function for HTC steroid response.

d) We are trying to develop reliable methods to enucleate cells so that hybrids can be made between either the nucleus alone or to the enucleated cytoplasm, and the whole cells. These would be useful in trying to dissect the sources of regulatory elements (Gopalakrishnan).

3) Other Studies on Steroid Receptors

a) Steroid receptors have been found to be present in both a number of steroid-unresponsive HTC variants and in several lines of steroid-insensitive leukemic cells (Lippman). This paradox, the presence of receptor which appears in all the ways tested so far to be normal in steroid insensitive cells, we propose to investigate further (Lippman).

b) Our studies on human acute lymphoblastic leukemia have been extended to acute myelogenous leukemic blasts (Lippman, Perry).

c) We have begun a long-term study of a series of steroid receptors in human breast carcinoma (Tormey).

d) A study on the ontogeny of steroid receptors in chick neural retina has been carried out, showing that early embryos, which do not induce for glutamine synthetase, nevertheless contain high levels of receptor (Wiggert, Cheder, Lippman).

4) Serum-Free Cell Line: The amino acid requirements have been determined, and various physiological parameters assessed. These cells should prove useful in future hybrid work.

5) Pilot Studies suggest that dexamethasone plus IUDR can rescue RNA viruses from HTC cells (Yang).

6) Cell-free Protein Synthesis: Studies were completed on poly dT effects on translation. Pilot experiments with a system derived from wheat germ look promising.

Significance to Cancer Research: We continue to be concerned how animal cells' genes are regulated because we hope that knowledge in this area will be important to controlling cancer. Control of growth and cell responses to steroids seem good ways to approach this problem. We also continue to try to apply our results to clinical situations. The studies begun on breast cancer patients, derived directly from our basic research interest, will eventually provide data about the biology of the disease and assist in therapeutic decisions as well.

Proposed Course of Research:

1) Analysis of the genetics and biochemistry of growth control in cultured cells.

2) Use of hybrids to study control of gene expression directly extending the work described above.

3) Continued work on steroid receptors and what they do in cells and human tumors.

4) Continued study of cell-free protein-synthesis.

Honors and Awards: Invited speaker, 4th International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, Japan "Differentiation and Control of Malignancy of Tumor Cells" November, 1973.

Invited speaker and session chairman, 1973 Gordon Conference on Hormone Action.

Invited speaker and session chairman, Symposium on "Gene Expression and Carcinogenesis in Cultured Liver" to be held May 1974 at UCLA.

Publications:

1. Levisohn, S.R., and Thompson, E.B.: Contact inhibition and gene expression in HTC/L cell hybrid lines. J. Cell. Physiol. 81: 225-232, 1973.

2. Thompson, E.B.: Temporal aspects of macromolecular synthesis in eukaryotic cells. In Urquhart, J. and Yates, F.E. (Eds.): Temporal Aspects of Therapeutics. Alza Conference Series Vol. 2, 1973. Plenum Press, New York, pp. 71-90.

3. Lippman, M.E., Halterman, R.H., Leventhal, B.C., Perry, S., and Thompson, E.B.: Glucocorticoid-binding proteins in human acute lymphoblastic leukemic blast cells. J. Clin. Invest. 52: 1715-1725, 1973.

4. Lippman, M.E., and Thompson, E.B.: Differences between cytoplasmic glucocorticoid-binding proteins shown by heterogeneity of nuclear acceptor sites Nature 246: 352-355, 1973.

5. Lippman, M.E., and Thompson, E.B.: Drug combinations in malignant disease. The Lancet I 7813: 1198, 1973.

6. Yang, S.S., Lippman, M.E., and Thompson, E.B.: Transfer ribonucleic acid in hepatoma tissue culture cells I. Induction in vivo by dexamethasone phosphate of phenylalanine-accepting activity. Endocrinology 94: 254-261, 1974.

7. Lippman, M.E., Yang, S.S., and Thompson, E.B.: Transfer ribonucleic acid in hepatoma tissue culture cells II. Dexamethasone phosphate induction of $tRNA^{phe}$ analyzed under cell-free conditons. Endocrinology 94: 262-266, 1974.

8. Thompson, E.B., and Lippman, M.E.: Mechanism of action of glucocorticoids. Metabolism 23: 159-202, 1974.

9. Thompson, E.B., Benedict, W.F., Owens, I.S., and Nebert, D.W.: Aryl hydrocarbon hydroxylase and tyrosine aminotransferase activities in somatic-cell hybrids derived from hepatoma tissue culture HTC (rat) cells and 3T3 (mouse) benzo[a]pyrene-resistant cells. Biochem. Biophys. Res. Commun. 56: 605-610, 1974.

10. Lippman, M.E., and Thompson, E.F.: The role of transcortin in glucocorticoid mediated enzyme induction: tyrosine aminotransferase induction in hepatoma tissue culture cells. J. of Steroid Biochemisty. In Press.

11. Lippman, M.E., and Thompson, E.B.: Steroid receptors and the mechanism of the specificity of glucocorticoid responsiveness of somatic cell hybrids between hepatoma tissue culture cells and mouse fibroblasts. J. Biol. Chem. In Press.

Serial No. NCI-366
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biosynthesis and assembly of intracellular structural components

Previous Serial No.: Same

Principal Investigator: Edward L. Kuff

Other Investigators: Kira K. Lueders, Shoshona Segal, Dante Marciani, Samuel H. Wilson

Cooperating Units: John D. Minna, Lab. of Biochem. Genetics, NHLI

Man Years: Total: 4
Professional: 3
Other: 1

Project Description:

Objectives: To study the synthesis and transport of individual macromolecules and their assembly into complex intracellular components; to study the process of neoplastic transformation in terms of these coordinated functions.

Methods Employed: Cell fractionation by differential and density gradient centrifugation, isolation and gradient fractionation of nucleic acids and nucleoproteins, electron microscopy of isolated nucleoproteins, *in vivo* and *in vitro* incorporation of isotopically-labelled precursors into protein and nucleic acid, chromatographic, immunochemical and electrophoretic characterization of specific proteins, tissue culture of normal and transformed cells, nucleic acid hybridization, immunofluorescent staining.

Results: Studies during the past year were concerned primarily with intracisternal A-particles.

1. The major structural protein of mouse intracisternal A-particles has a molecular weight of 73,000 (P73). The particles also contain lesser amounts of proteins with molecular weights of about 110,000, 46,000 and 32,000 daltons. D. Marciani had previously shown that all of these components exhibited a common antigenic reactivity. He has now analyzed CNBr and tryptic peptides derived from the various sized proteins. P73, P46 and P32 all contain a large proportion of common peptides, indicative of very extensive

sequence homology in spite of differences in molecular weight. The possibility of repetitive or partially repetitive sequences is suggested.

2. Biosynthesis of the main structural protein (P73) has been studied in cultured neuroblastoma cells by K. Lueders. In the most thoroughly characterized cell line, synthesis of P73 represents approximately 1.5% of the total cell protein synthesis. This proportion does not change markedly when the cells pass from log growth to a confluent state or when they are induced, by treatment with IUdR and dexamethasone, to simultaneous synthesis of extracellular C-type virus particles. Label appears within P73 within two minutes after radioactive amino acids are added to the medium. Pulse-chase and inhibitor studies indicate that the pool of unassembled (soluble) P73 is small. Once labelled, the A-particle protein turns over more rapidly then the average cell protein. A thorough search has failed to reveal extracellular particles, soluble particle protein, or antigenic activity; it seems therefore that the particles are turned over intracellularly and perhaps in their intracisternal position. This suggestion is of interest in light of recent electron microscopic evidence that very few particles are completely free within the cisternal space, most of them retaining a stalk-like connection with the endoplasmic reticulum membrane. It is possible that the particles undergo a continuous cycle of budding and resorption rather than the unidirectional secretory-like process which has been assumed.

3. A-particles contain 4-5% RNA. The RNA prepared from myeloma-derived A-particles has been studied by S. Segal. No 60-70S RNA was detected. About 12% of the RNA was retained on an oligo(dT)- cellulose column under conditions which favor binding of poly(rA)-containing molecules. Quantitive hybridization with radiolabelled poly(dT), prepared by S. Wilson, indicate an average poly(rA) content of 5% for the column-isolated RNA fraction. On gradient analysis, this material was heterogeneous with peak sedimentation values in the 9-13S range. Dr. P. Leder prepared a radioactive DNA probe against the poly(rA)-containing RNA, using avian myeloblastosis virus reverse transcriptase. The kinetics of annealing of the RNA with its probe revealed a degree of sequence heterogeneity consistent with the presence of multiple types of messenger RNA molecules. The probe did not react with 70S RNA from murine leukemia virus (Rauscher).

4. Rabbit antiserum prepared against isolated P73 was used to develop a specific and apparently very sensitive immunofluorescent staining procedure for A-particle containing cells.

Significance for Cancer Research: Intrascisternal A-type particles are observed regularly in both early and late mouse embryos, but very rarely in normal cells of adult animals. Particle synthesis can be re-activated, often at highly amplified levels, in cells transformed by a variety of agents (viruses, radiation, chemical carcinogens). Our studies are aimed at understanding the regulation of A-particle synthesis and determining whether their expression is biologically significant in neoplastic transformation.

Proposed Course of Research: Experiments will be designed to (a) test for possible *in vivo* function of the particle-associated DNA polymerase; (b) characterize the information content of the RNA by further hybridization studies and by examination of the protein products for which it might code in a cell-free synthesizing system; and (c) localize the intracellular site of synthesis of the particle proteins and examine protein transport to the sites of particle assembly on the membrane surfaces.

Publications:

1. Wilson, S. H., Kuff, E. L., Bohn, E. W., Lueders, K. K., and Matsukage, A: DNA polymerase in association with intracisternal A-type particles, in DNA Synthesis *In Vitro*, (ed.): R. D. Wells and R. B. Inman, University Park Press, Baltimore, Md., 1973, pp 361-367.

2. Marciani, D., and Kuff, E. L.: Isolation and partial characterization of the internal structural proteins from intracisternal A-particles. *Biochemistry* 12: 5075-5083, 1973.

3. Wilson, S. H., Bohn, E. W., Matsukage, A., Lueders, K. K., and Kuff, E. L.: Studies on the relationship between DNA polymerase activity and intracisternal A-type particles in mouse myeloma. *Biochemistry*. In press.

4. Minna, J. D., Lueders, K. K., and Kuff, E. L: Expression of genes for intracisternal A-particles antigen in somatic cell hybrids. *J. Nat. Cancer Inst.*, In press.

Serial No. NCI-5214
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Gene replication and repair in mammalian cells

Previous Serial No.: Same

Principal Investigator: Samuel H. Wilson

Other Investigators: A. Matsukage, J. D. Minna, T. Marshall, E. L. Kuff,
B. K. Schrier, K. Stromberg and A. F. Gazdar

Cooperating Units: National Heart & Lung, National Institute of Child Health

Man Years: Total: 3
Professional: 2
Other: 1

Project Description:

Objective: The broad objective of this research program is to understand mechanisms and regulation of DNA synthesis in mammalian cells; our initial approach has been investigation of DNA synthesizing enzymes in mouse tissue.

Our previous analysis of the DNA polymerase content of mouse myeloma MOPC-104E revealed five distinct enzymes that possessed relatively abundant activity. Two of these enzymes appeared to correspond to DNA polymerases well characterized in other tissues and a third enzyme exhibited properties similar to those of the mouse type-C virus-associated DNA polymerases. The other two enzymes had not been clearly recognized previously. During the past year other laboratories have also detected these two new enzymes and areas of investigation concerning their properties and biological roles are emerging.

One of these two newly recognized polymerases possessed the most abundant activity among the mouse myeloma DNA polymerases. We designated this enzyme DNA polymerase III, and during the past year undertook a more thorough investigation of its properties and relationship to other DNA polymerases. This enzyme was chosen for further investigation because we believe it may play an important role in the regulation of gene expression in mammalian cells. Our results suggest that DNA polymerase III is a cellular enzyme located in the cytoplasmic membranes that is capable of making DNA with base sequences complementary to those in cytoplasmic m-RNA. In this regard, DNA polymerase III differs from the other cellular DNA polymerases tested.

Methods Employed: Procedures were developed for extraction and fractionation of DNA polymerase activities in mouse tissues. The procedures involved subcellular fractionation, column chromatography and assays for DNA polymerase activities using different types of reaction conditions. Cell fractionation procedures and characterizations of reaction products were performed using conventional techniques of centrifugation rate-zonal centrifugation, scintillation spectrophotometry and various types of chromatography.

Major Findings:

1. Purification and Characterization of Mouse Myeloma MOPC-104E DNA Polymerase III.
DNA polymerase III in an extract prepared from unfractionated myeloma was purified to apparent homogeneity as judged by polyacrylamide gel electrophoresis. The molecular weight of the purified enzyme was 1.5-2 X 10^5 daltons estimated by electrophoretic mobility and sedimentation rate. The purified enzyme possessed no detectable RNA polymerase, DNase, DNA endonuclease or nucleoside diphosphokinase activities. However, it did possess abundant ribonuclease activity that hydrolyzed the RNA moiety of RNA·DNA hybrids, but not free RNA. The DNA polymerase activity of the enzyme required a template for direction of which deoxynucleotide to incorporate, and a 3'-OH group-containing primer for initiation of incorporation. Reduced sulfhydryl groups were required for DNA polymerase activity, and the activity was inhibited by o-phenanthroline, suggesting that the polymerase is a zinc metaloenzyme. The DNA polymerase activity was directed by several synthetic RNA and DNA templates including poly(rA), poly(dA) and poly(dC), but was inactive with calf thymus DNA, poly d(A-T) and poly(rC) as template. The mechanism of action of the DNA polymerase was different in reactions directed by poly(rA)·$(dT)_{12-18}$ in the presence of Mn^{2+} than in reactions directed by poly(dA)·$(dT)_{12-18}$ in the presence of Mg^{2+}.

2. Detection of DNA Polymerase III in Adult BALB/c Mouse Liver: An enzyme with chromatographic and sedimentation properties virtually identical with those of the myeloma DNA polymerase III was detected in an extract prepared from the livers of 12-14 week old BALB/c female mice. This liver enzyme was distinguished from two previously recognized cellular enzymes present in liver, the high molecular weight (6S) cytoplasmic high speed supernatant fraction DNA polymerase and the low molecular weight (3.4S) DNA polymerase. As in the case of the myeloma DNA polymerase III, the liver enzyme was active with poly(rA)·oligo$(dT)_{12-18}$, but not with activated calf thymuc DNA or poly d(A-T).

3. Use of Natural RNA as Template by DNA Polymerase III. Highly purified DNA polymerase III from mouse liver and myeloma was 10-40% as active with mouse liver m-RNA·$(dT)_{12-18}$ as template-primer as with poly (rA)·$(dT)_{12-18}$. The m-RNA-directed incorporation was dependent upon $(dT)_{12-18}$ and was reduced when one of the four deoxynucleotides was omitted. Nearest neighbor analysis

of the product from reactions containing m-RNA·$(dT)_{12-18}$ and alpha [32 P] deoxynucleoside-5'-triphosphates as substrates revealed that dAMP, dCMP, dGMP and dTMP were present in the DNA product.

4. Inhibition of 2.5S Myeloma MOPC-104E DNA Polymerase by Sulfhydryl-Blocking Reagents. Activity of a 2.5S mouse myeloma DNA polymerase measured with either poly(rA) or poly(dA) as template did not require sulfhydryl-reducing reagents, but was inhibited by several reagents that react with sulfhydryl groups. Enzyme activity was much more sensitive to inhibition by p-hydroxymercuribenzoate (PHMB) than by the sulfhydryl-aklyating reagents, N-ethylmaleimide and iodoacetamide. The PHMB inhibition appeared to involve the mercurial portion of the PHMB molecule because $HgCl_2$ was an equally effective inhibitor, whereas p-hydroxybenzoate had little effect upon enzyme activity. The PHMB inhibition was reversed by an equal concentration of the sulfhydryl-reducing reagent, dithiothreitol.

5. Stimulation of DNA Polymerase Activity by the Combination of P-Hydroxymercuribenzoate and Dithiothreitol. The combination in reaction mixtures of 1 mM dithiothreitol (DTT) and 1 mM P-hydroxymercuribenzoate (PHMB) resulted in a 10-fold stimulation in the activity of a 2.5S mouse myeloma DNA polymerase. The stimulation, first detected using poly(rA)·oligo(dT) as template-primer, was also observed with several other template-primers. In addition, a comparable stimulation was observed in the poly(rA)-directed activity of the 3.4S DNA polymerase from mouse liver, murine leukemia virus DNA polymerase and E. coli DNA polymerase I, while no stimulation was observed with the intracisternal A-particle DNA polymerase or the 7.9S mouse myeloma DNA polymerase. The stimulatory effect of DDT:PHMB appeared to to involve the mercurial portion of the PHMB molecule because $HgCl_2$ substituted for PHMB whereas p-hydroxybenzoate did not.

6. Studies on Intrasisternal A-Particle Associated DNA Polymerase Activity in Neuroblastoma C-1300 Cells. Using a simplified new procedure for isolation of A-particles (developed by Dr. E. L. Kuff and co-workers), the subcellular distribution of the A-particle DNA polymerase activity in neuroblastoma cells was determined. Aspects of this work are described in Annual Report NCI-366, prepared by Dr. E. L. Kuff.

7. Oncornavirus Expression in Human x Mouse Hybrid Cells Segregating Mouse Chromosomes. In collaboration with Dr. J. D. Minna (NHLI) and Dr. A. F. Gazdar (NCI), human x mouse hybrid clones obtained by fusing transformed human (VA2) cells with embryonic mouse brain cells were tested for their ability to spontaneously express type-C virus particles. It had been previously shown that these hybrid cells preferentially retained human chromosomes while mouse chromosomes were lost. The culture fluid from one cell line was found to contain type-C particle markers in abundance, and typical budding immature type-C particles were observed in the cells by electron microscopy. In contrast, no particle markers were detected in the culture fluid from parental cells and several other hybrid cell lines. Subclones of the virus positive cell line continued to lose mouse chromosomes and were found to vary more than 100-fold in their culture fluid DNA polymerase activity. The

hybrid cell virus, termed HMV1, banded between 1.14 and 1.16 g/ml, possessed viral group-specific (gs) antigens, exhibited B-tropic host range for replication in mouse embryo cells (MEC) but did not replicate in hyman cells when directly applied. The virus did not transform mouse cells but was able to rescue the defective murine sarcoma virus from sarcoma positive helper virus negative cells. Activity of the DNA polymerase associated with HMV1 was similar to the activity of Rauscher murine leukemia virus (MuLV) DNA polymerase in its preference for poly(rA) over poly(dA) as a template, use of endogenous template, detergent requirement and inhibition by antiserum directed against MuLV DNA polymerase. The results demonstrate that human x mouse hybrid cells segregating mouse chromosomes can spontaneously express type-C viruses with several properties characteristic of a murine leukemia virus.

8. Methods for Measurement of Radioactive DNA and Application to Assays for DNA Polymerase Activity. In collaboration with Dr. B. K. Schrier (NICHD), methods for collection and counting of ^{3}H-labeled DNA on nitrocellulose and glass fiber filters were investigated. The highest counting efficiencies were observed using glass fiber filters, NCS for dissolving DNA, and a toluene scintillation mixture for counting. However, glass fiber filter even with large amounts of co-precipitant albumin, failed to collect all of lo molecular weight (approx. 185,000 daltons) DNA. Thus, in many applications nitrocellulose filters are more advantageous.

Significance to Cancer Research: Detailed knowledge of the mechanisms and regulation of DNA synthesis is important in understanding the molecular biolog of cancer. Many approaches are being used to investigate DNA synthesis in normal and neoplastic cells, and advances in our understanding on all levels will prove useful in preventing, treating and ultimately controlling cancer. Activity of DNA synthesizing enzymes is necessary for maintenance of a rapid rate of cell division, and in some cases they may play an important role in the development of the transform state.

Proposed Course of Research:

1. To determine the DNA polymerase content of murine tissues in various stages of growth regulation, using the methods of systematic fractionation and detection of DNA polymerases described above. Cells in the process of active replicative DNA synthesis and cells that are inactive in DNA synthesis will be studied, as well as cells in the process of repairing damaged DNA.

2. To further purify the forms of murine myeloma DNA polymerases that have been isolated and determine their biochemical, immunological and physical properties.

3. To continue investigation of oncornavirus and DNA polymerase expression in human mouse hybrids.

4. To solubilize, purify and characterize the A-particle-associated DNA polymerase.

Honors and Awards: None

Publications:

1. Stromberg, K., Gantt, R., and Wilson, S. H.: Structural studies on avian myeloblastosis virus. Conditions for isolation and biochemical characteristics of the core component. Biochim. Biophys. Acta 304: 1-11, 1973.

2. Schrier, B. K., and Wilson, S. H.: Investigation of methods for measurement of radioactivity in tritiated DNA and applications to assays for DNA polymerase activity: Anal. Biochem. 56: 196-207, 1973.

3. Matsukage, A., Bohn, E. W., Wilson, S. H.: Multiple forms of DNA polymerase in mouse myeloma: Proc. Nat. Acad. Sci. USA: 771: 578-582, 1974.

4. Wilson, S. H., Bohn, E. W., Matsukage, A., Lueders, K. K., and Kuff, E. L.: Studies on the relationship between deoxyribonucleic acid polymerase activity and intracisternal A-type particles in mouse myeloma: Biochemistry 13: 1974

5. Bohn, E. W. and Wilson, S. H.: Measurement and properties of the DNA polymerase activity associated with intracisternal A-particles: Can. Res. In press, 1974.

6. Minna, J., Gazdar, A. G., Iverson, G. M., Marshall, T., Stromberg, K., and Wilson, S. H.: Oncornavirus expression in human-mouse hybrid cells segregating mouse chromosomes: Proc. Nat. Acad. Sci. USA.In Press, May 1974.

7. Bohn, E. W., Matsukage, A. and Wilson, S. H.: Stimulation of DNA polymerase activity by the combination of P-hydroxymercuribenzoate and dithiothreitol: Biochem. Biophys. Res. Commun. In press, 1974.

Serial No. NCI-5217
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of mechanisms of information transfer between cells of nervous system origin

Previous Serial No.: Same

Principal Investigator: Samuel H. Wilson

Other Investigators: M. Nirenberg, R. Pertel, B. K. Schrier, E. J. Thompson

Cooperating Units: Laboratory of Biochemical Genetics, National Heart and Lung Institute

Man Years: Total: 1/20
Professional: 1/20
Other: 0

Project Description:

Objectives: To develop techniques and procedures for investigation of communication between cells in the nervous system.

Methods Employed: Layer culture of primary mouse cells, neuroblastoma C-1300 cells, and the free-living nematode, C. elegans, was performed in plastic petri dishes in conventional culture media. Assays based upon measurement of enzyme activities and the principal of isotope dilution were developed, and used to determine the amounts of neurotransmitters present in the cultured cells.

Major Findings: None

Significance to Cancer Research: This research project has produced a number of new techniques (documented in this and previous reports) for study of gene expression in mammalian cells, and thus, has made an important contribution to basic cell biology. Ultimately, a more thorough understanding of gene expression in mammalian cells will provide guides to control of malignan growth and thus will provide new insight into practical cancer therapy and prevention.

Honors and Awards: None

Publications:

1. Thompson, E. J., and Wilson, S. H., Schuette, W. H., Whitehouse, W. C., and Nirenberg, M. W.: Measurement of the rate and velocity of movement by single heart cells in culture. Amer. J. Card. 32: 162-166, 1973.

2. Pertel, R. and Wilson, S. H.: Histamine content of the nematode, *Caenorhabditis Elegans*.: Comp. Gen. Pharm., In Press, 1974.

3. Schrier, B. K., Wilson, S. H. and Nirenberg, M.: Cultured cell systems and methods for neurobiology. In Methods in Enzymology, (Fleischer, S., Packer, L. and Estabrook, R. W., eds.) 1974, 32, In Press.

Serial No. NCI-945
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of the Factors Regulating the Synthesis of Collagen during Embryonic Development and Growth of Cultured Fibroblasts

Previous Serial Number: Same

Principal Investigator: Beverly Peterkofsky

Other Investigators: Thomas Blanck, M. D., Research Associate, Carlotta Evans, D.D.S., NIH-ADA Fellowship, Guest worker.

Cooperating Units: None

Man Years: Total: 4.6
Professional: 2.8
Other: 1.8

Project Description:

Objectives: To elucidate the basic mechanisms of collagen synthesis and secretion and to investigate the factors which regulate these processes in cultured fibroblasts, both normal and transformed, and during embryonic development.

Methods Employed: Proteins of whole chick embryos, isolated bones of these embryos, or cell-free systems derived from these bones or cultured fibroblasts are labeled with ^{14}C-proline. The proteins are precipitated with trichloroacetic acid, redissolved and then assayed for radioactivity in collagen and noncollagenous proteins by a method involving specific digestion of collagen in a mixture of proteins using highly purified collagenase. This method was developed in our laboratory and is described in a previous report (1968-9). The relative rate of collagen synthesis can be calculated from data obtained by this method and the specific rate of synthesis in tissues or cells in calculated by determining the DNA content and expressing the rate as cpm/μg DNA. The proteins of the cell and medium fractions of cultured fibroblasts are analyzed separately in order to study secretion. Morphological changes in cultured cells are recorded by polaroid photomicroscopy.

Cell-free incorporation of ^{14}-C-proline is studied with a system consisting of membrane-bound or free polysomes derived from chick embryo bone tissue and soluble factors derived from chick embryo liver, a tissue which synthesizes very little collagen, so that variations in collagen-synthesizing polysomes of a tissue may be studied independently of possible variations in the soluble fraction.

To determine the level of proline hydroxylation in collagen of cells or tissues labeled with radioactive proline, collagenase digests are hydrolyzed with 6N HCl and radioactive proline and hydroxyproline in the hydrolyzates are measured by a specific radioassay. To measure the level of lysine hydroxylation, cells or tissues are labeled with radioactive lysine and hydrolyzed collagenase digests are chromatographed on Dowex-50 in 2N HCl, which separates lysine and hydroxylysine.

Prolyl hydroxylase is measured by tritium release from H^3-proline labeled unhydroxylated collagen prepared by incubating chick embryo frontal bones with H^3-proline in the presence of the iron chelator αα-dipyridyl, which inhibits hydroxylation.

Cell lines used in these studies: Chick embryo fibroblasts prepared by digesting frontal bones from 15 day chick embryos with crude collagenase and culturing the released cells. Subcultures in the second to fifth passage are used.

L-929- a line of mouse embryo fibroblasts established about 30 years ago by use of a chemical carcinogen.

Balb-3T3- a contact inhibited line of mouse embryo fibroblasts isolated by Todaro and Aaronson.

Balb-3T6 and 3T12- lines of mouse embryo fibroblasts derived from the same pool of cells as 3T3 but non-contact inhibited and tumorigenic.

Ki-3T3- a line established by Aaronson by transformation with Kirsten sarcoma virus. These cells do not produce viral particles unless superinfected with murine leukemia virus.

Embryonic and adult human diploid fibroblasts-purchased commercially.

Major Findings:
1. Quantitation and localization of prolyl hydroxylase.
We had previously shown that almost all of the collagen synthetic activity of connective tissue occurs on polysomes bound to endoplasmic reticulum and that proline residues in unhydroxylated collagen synthesized on these polysomes can subsequently be hydroxylated by endogenous prolyl hydroxylase in the presence of the required cofactors, ascorbate, Fe^{++} and α-ketoglutarate. We are now in the process of determining the amount of prolyl hydroxylase in these endoplasmic reticulum vesicles to determine whether most of the enzyme activity resides there as well. In order to assure that all of the enzyme activity in particulate fractions is recovered during separation of subcellular fractions, we have studied the requirements for stability of the enzyme. It was found that when the small

molecule fraction was removed from solubilized prolyl hydroxylase in a crude extract, the enzyme was completely inactivated upon heating at 50° for 5 min, while it was completely stable in the presence of ascorbate, α-ketoglutarate and iron.

II. Regulation of Collagen Secretion by Ascorbic Acid

A. Studies using chick embryo fibroblasts have been continued and we have obtained further evidence that stimulation of collagen secretion by ascorbate is directly related to the level of proline and/or lysine hydroxylation. This evidence may be summarized as follows:

1. αα-dipyridyl inhibits both proline and lysine hydroxylation by chelating Fe^{++}, a hydroxylase cofactor, and collagen secretion is inhibited when this chelator is added to chick embryo cell cultures. If ascorbate is added in addition to αα-dipyridyl, there is no increase in the amount of secretion, suggesting that once secretion is inhibited by blocking hydroxylation, ascorbate exerts no other stimulatory effect.

2. The level of hydroxylation of proline and lysine residues in collagen was varied either by adding varying concentrations of ascorbate or of αα-dipyridyl during a 2 hr labeling period with ^{14}C-prolice. The extent of proline hydroxylation was measured by analyzing radioactive proline and hydroxyproline in a hydrolyzed collagenase digest. The percent of potential hydroxyproline residues actually hydroxylated was plotted versus percent secretion and it was found that points obtained from either the αα-dipyridyl or ascorbate experiments fell on the same curve. The curve indicated that secretion in the 2 hr labeling period remained at 20%, from complete unhydroxylation up to the point where 60% of the potential hydroxyprolines were hydroxylated. After that point, secretion increased linearly with hydroxylation. These results provide further evidence that ascorbate stimulates secretion by virtue of its ability to act as a cofactor for either prolyl or lysyl hydroxylase or both, in cells. Furthermore, others have reported that the collagen helix is much less stable when the molecule contains no hydroxyproline. In view of this, our results may also indicate that only a specified number of hydroxyproline residues play a role in helix stabilization and that secretion is directly proportional to the amount of helix structure stabilized by these residues. This further implies that hydroxylation of proline residues probably occurs in a linear sequence rather than in a random fashion.

B. The level of proline hydroxylation and the percent collagen secretion was measured in contact inhibited 3T3 and transformed Ki-3T3 during various stages of growth from early log to stationary phase by labeling cell proteins with ^{14}C-proline in the presence and absence of ascorbate. It was found that in the absence of ascorbate, collagen from 3T3 cells showed a very low level of proline hydroxylation but complete hydroxylation in the presence of the cofactor. In the transformed cells, however, hydroxylation was 80-90% complete even in the absence of the vitamin. In agreement with out observation in chick embryo cells that hydroxylation and the extent of secretion are directly related, the transformed cells showed almost no dependence on ascorbate for collagen secretion while 3T3 showed a marked dependence. Preliminary experiments indicate that the transformed cells do not contain ascorbate, therefore some other compound must be acting as a cofactor for prolyl hydroxylase in these cells and this compound must be present in much lower levels in the parent line.

III. Effect of Dibutyryl cAMP (DBcAMP) on the Growth, Morphology and Rate of Collagen Synthesis in Ki-3T3 Cells.

A. The maximum increase in the relative rate of collagen synthesis requires at least 3 days of growth in the presence of DBcAMP, although a two fold increase can already be observed after 20 hours of exposure.

B. The effect of DBcAMP appears to be specific. At equimolar concentrations, sodium butyrate, DBcGMP, and cAMP did not increase the relative rate of collagen or affect the growth or morphology of Ki-3T3 cells.

C. Growth, morphology and collagen synthesis were examined at concentrations of DBcAMP ranging from 0.1 to 1.0 mM. The following observations were made:

1. At 0.1 mM growth was not inhibited at all but morphological changes could already be discerned and collagen synthesis had increased slightly.

2. Changes in morphology appeared to have reached a maximum at 0.5 mM while growth was still not completely inhibited and the relative rate of collagen synthesis was still increasing at this concentration of the nucleotide.

D. DBcAMP had no effect on collagen synthesis in the parent 3T3 cells.

These results suggest that the properties of these transformed cells such as altered morphology, increased growth rate and decreased collagen synthesis as compared to the parent 3T3 cell line can be reversed to varying degrees within the same concentration range of DBcAMP, although each property may show a slightly different dose-response curve. The effects are probably due to an increase in the intracellular concentration of cAMP and thus the parent line, 3T3, which has been shown by Pastan and others to contain relatively high concentrations of the cyclic nucleotide, does not respond.

IV. The Toxicity of Ascorbate Towards Cultured Cell Lines.

This project arose as a result of observations made during our studies on the effect of ascorbate on collagen secretion. It was observed that some cell lines, such as chick embryo fibroblasts and Balb 3T3 fibroblasts were killed at a concentration of 0.25 mM ascorbate while other lines were not. Further investigation of this phenomenon has shown that contact inhibited cells such as chick embryo fibroblasts, human diploid fibroblasts, both embryonic and adult, and Balb 3T3 are either killed or have their growth inhibited by concentrations of ascorbate above 0.05 mM. The transformed synthesizing polysomes of a tissue may be studied independently of possible variations in the soluble fraction.

To determine the level of proline hydroxylation in collagen of cells or tissues labeled with radioactive proline, collagenase digests are hydrolyzed with 6N HCl and radioactive proline and hydroxyproline in the hydrolyzates are measured by a specific radioassay. To measure the level of lysine hydroxylation, cells or tissues are labeled with radioactive lysine and hydrolyzed collagenase digests are chromatographed on Dowex-50 in 2N HCl, which separates lysine and hydroxylysine.

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Cell lines used in these studies: Chick embryo fibroblasts prepared by digesting frontal bones from 15 day chick embryos with crude collagenase and culturing the released cells. Subcultures in the second to fifth are used passage.

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Balb-3T3- a contact inhibited line of mouse embryo fibroblasts isolated by Todaro and Aaronson.

Balb-3T6 and 3T12- lines of mouse embryo fibroblasts derived from the same pool of cells as 3T3 but non-contact inhibited and tumorigenic.

Ki-3T3- a line established by Aaronson by transformation with Kirsten sarcoma virus. These cells do not produce viral particles unless superinfected with murine leukemia virus.

Embryonic and adult human diploid fibroblasts-purchased commercially.

Major Findings:

1. Quantitation and localization of prolyl hydroxylase.

We had previously shown that almost all of the collagen synthetic activity of connective tissue occurs on polysomes bound to endoplasmic reticulum and that proline residues in unhydroxylated collagen synthesized on these polysomes can subsequently be hydroxylated by endogenous prolyl hydroxylase in the presence of the required cofactors, ascorbate, Fe^{++} and α-ketoglutarate. We are now in the process of determining the amount of prolyl hydroxylase in these endoplasmic reticulum vesicles to determine whether most of the enzyme activity resides there as well. In order to assure that all of the enzyme activity in particulate fractions is recovered during separation of subcellular fractions, we have studied the requirements for stability of the enzyme. It was found that when the small line Ki-3T3 is completely resistant to ascorbate toxicity up to 0.1 mM and shows greater survival than 3T3 at higher concentrations. The spontaneous transformants 3T6 and 3T12 are also much less susceptible to ascorbate toxicity than 3T3. The vitamin does not prevent attachment of cells but appears to cause lysis of attached cells. Ascorbate appears to act directly on the cells rather than causing reduction of some component of the culture medium since preincubation of medium with acscorbate for a time long enough to allow degradation of the ascorbate results in a loss of toxicity. The varying sensitivity of contact-inhibited cells compared to transformed cells and the lytolylic action of ascorbate suggests that the cell membrane is being affected.

Proposed Course of Research

I. The amount of prolyl hydroxylase in subcellular fractions of bone tissue will be quantitated. If most of the activity is found to be in endoplasmic reticulum vesicles, fractionation of vesicles will be undertaken to determine whether the enzyme is bound to membranes or is intracisternal. In addition, the location of other enzymes of collagen biosynthesis such as lysyl hydroxylase and the glycosyl transferases will be determined.

II. A. The relationship between lysine hydroxylation and ascorbate concentration will be examined in chick embryo cells to determine whether this function is also affected as well as proline hydroxylation. Collagen secreted during maximum and minimun hydroxylation will be examined to determine whether hydroxylation occurs in a specific linear sequence.

B. The identification of the hypothetical hydroxylase cofactor present in transformed cells will be pursued.

III. Further studies will be carried out to determine the mechanism by which DBcAMP increases collagen synthesis in Ki-3T3 cells.

IV. Experiments will be carried out to determine the mechanism by which ascorbate kills contact inhibited cells and the specificity of the effect. Additional cell lines will be tested to verify our theory that transformed cells are less sensitive to ascorbate toxicity.

Significance to Biomedical Research and the Program of the Institute:

Determining the precise mechanism by which vitamin C affects collagen synthesis should lead to a more accurate use of this vitamin in general and especially in cases where surgery has been used extensively, as in cancer patients. Our studies with dibutyryl cyclic AMP are aimed at determining the extent to which this chemical reverses cell transformation.

Publications:

1. Diegelmann, R. F., Bernstein, L., and Peterkofsky, B.: Cell-free collagen synthesis on membrane-bound polysomes of chick embryo connective tissue and the localization of prolyl hydroxylase on the polysome-membrane complex. J. Biol. Chem. 248: 6514-6521, 1973.

Serial No. NCI-5215
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biological and ultrastructural studies on cell cooperation in the immune response.

Previous Serial: Same

Principle Investigator: Emma Shelton

Other Investigators: Jan Orenstein

Man Years: Total: 3
Professional: 2
Other: 1

Project Description:

Objective: To examine the interaction of lymphocytes and macrophages cooperating in the immune response and specifically:

A. To develop a method for the culture of small numbers of cells that will differentiate into antibody-producing cells.

B. To examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen.

Methods and Major Findings

1. A word about horseradish-peroxidase (HRP) and replica technique: In initiating this project, we believed that HRP would be the ideal antigen to use since it can be visualized in light and electron microscopy, but unfortunately, it is a very poor immunogen and we were unable to get predictable differentiation when sensitized cells were exposed to antigen. We have turned to sheep erythrocytes as an antigen whose characteristics are well known.

We plan to use the ferritin-labeled anti-HRP antibody that we made last year to follow the retention of HRP on macrophage surfaces using the replica technique.

We have learned a lot about the replica technique during the past year. For preserving the detail of cell surfaces, critical point drying is vastly superior to freeze-drying. We hope to produce some meaningful replicas during the coming year.

2. Differentiation of small populations of lymphocytes into antibody-producing cells:

Lympnocytes and macrophages are notoriously difficult to culture, thus, most immunologists use 10^6 or 10^8 cells in their culture experiments. We want to look at individual cells and 10^6 cells is a jungle, so we have been plugging away with 10^4 cells.

Cells were cultured in diffusion chambers implanted by laparotomy into mice. The cells were obtained by lavage of the undisturbed peritoneal cavity of normal and sensitized mice with 1 ml of buffered saline. There were exposed to antigen in the test tube, droplets are put into the chambers put into mice within a period of two hours.

The trick to growing small numbers of cells is to use a "floating" filter inside the chamber. Three to 5 microliters droplets of fluid containing 20- 50 x 10^3 cells are place on the floating filter and the cells are allowed to settle and attach over a period of 15-30 minutes before the chambers are implanted into mice.

Both normal and sensitized cells have been grown in the presence and absence of antigen. The changes in the cell population with time has been followed by removing the chambers, staining the filters and examining them in the light microscope. The cells were examined at 6 hour intervals up to 72 hours and at 6 and 10 days.

About $^{1}/3$ to $^{1}/2$ of the cell clusters survive to differentiate. Differentiation occurs during 48-72 hours and at 6 days soluble antibody can be measured in the chamber fluid. Thus, we can look at early interactions knowing that they are among cells that will produce specific antibody.

During the first 24 hours there is severe attrition of the cell number, usually more striking in sensitized cells. Free sheep cell disappear from sensitized cell clusters after 6 hours, while in normal cell clusters they are still present for days. Between 30-36 hours there is a peak of mitotic activity and between 48 and 72 hours cell clusters are formed that are the hallmark of immunological differentiation. They consist of one or two macrophages, a few undifferentiated lymphocytes and varying numbers of large basophilic cells that suggest clones. The membrane morphology of the basophilic cells is so striking as to give the impression of intense activity to the static, fixed cell preparation. This impression of activity is imparted by the nature of the cytoplasmic protrusions extending from the cell membrane. Each basophilic cell has one or several pseudopodia that can extend for a distance two or three times the diameter of the cell and terminate in a ruffled fan. Most frequently these pseudopodia terminate in contact with another cell.

The plasma cells continue to increase so that after 6 days they become the principal cell and often reach astonishing numbers; mitotic figures are continually seen.

3. Scanning electron microscopy: The scanning electron microscope (SEM) has proved to be an exceedingly useful instrument for examination of membrane changes in these cell systems. We have spent most of our time with early interactions, 5, 10 and 20 minutes.

Direction-oriented corona or cytoplasmic processes: A new finding; lymphocytes and macrophages that are within one cell diameter of each other, extend a corona of cell processes that is absolutely oriented toward the neighboring cell. The corona is not present on separated cells. The direction-oriented processes extend out from the cell surface several times the distance of the microvilli that normally cover the cell. The processes are smooth and may terminate in a point or in several convolutions. With time the processes of the coronas of adjacent cells interdigitate and the cells come together in very close contact. The phenomenon is seen in both normal and sensitized cell populations.

In addition to the coronas, lymphocytes and macrophages extend single stout cytoplasmic processes that are also direction oriented. These tend to have blebs along their length-like buds on a twig. They may terminate in a sharp point or complex cluster.

Short-term interactions between normal and sensitized cells in the presence of antigen:

To study short-term interactions, 3 ul droplets containing cells and sheep erythrocytes (SRBC) were plated on Millipore filters and incubated at 37°C for intervals of 5, 10 and 20 minutes; fixed and prepared for light and scanning electron microscopy. For the moment we are interested in describing what we see. If the surface topography of normal cells incubated with SRBC is taken as a baseline, then it can be said that every membrane feature is exaggerated when sensitized lymphocytes and macrophages are incubated with SRBC. The coronas are seen on more cells and they extend over a longer distance. The cells come into very close contact with one another extremely rapidly. The SRBC are attached to many lymphocytes. Within 5 minutes, macrophages begin to engulf SRBC and (surprisingly) lymphocytes by surrounding them with a sheet of cytoplasm. The engulfment is a very local phenomenon that occurs without disturbing the morphology of the adjacent surface.

Proposed Course:

A. To develop a method for the culture of small numbers of cells that will differentiate into antibody-producing cells.

B. To examine by light microscopy and by transmission and scanning electron microscopy the early interactions of cells that are presented with antigen, and specifically to continue with scanning electron microscopy to:

Characterize and compare surface morphology of lymphocytes derived from the peritoneal cavity, thymus and lymph node.

Describe surface morphology of cells that have differentiated into plasma cells in diffusion chambers.

C. To prepare hybrid antibody to tag T and B cells for identification in SEM and to follow fate of these cells in differentiation in diffusion chambers.

D. To continue with replica technique and to look at retention of HRP on macrophage surfaces.

Honors and Awards: Associate Editor, Cancer Research

Publications: None

Serial No. NCI-379
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ultrastructural characterization of immunoglobulins, enzymes and other biomacromolecules.

Previous Serial No.: Same

Principal Investigator: Emma Shelton

Other Investigators: J. M. Orenstein

Cooperating Units: R. A. Lazzarini, Lab. Mol. Biol., NINDS
R. L. Switzer, Dept. Biochem, Univ. of Illinois, Urbana, Ill.
B. Babior, Thorndike Memorial Lab., Boston City Hospital, Boston, Mass.

Man Years: Total: 4
Professional: 3
Other: 1

Project Description:

Objectives: To relate the fine structure of macromolecules to their biochemical and physical properties, and to their function in biological systems.

Methods and Major findings:
1. Structure of Vesicular Stomatitis Virus (Orenstein, Lazzarini).

This virus contains a single-stranded RNA genome and five proteins, easily identified by SDS polyacrylamide gel electrophoresis. In order to determine the structure of these components and their locations in the intact virion, we are employing negative staining and transmission electron microscopy to examine preparations of virus from which the various proteins have been selectively deleted.

One protein appears to be located between the glycoprotein-containing membrane and the central coil of RNP while a second, possibly the RNA polymerase, seems to be loosely packed in the hollow of the RNP coil in the core of the virion.

The fine structure of the coiled RNP strand is of greatest interest to us because we have evidence that the ends of the rod-like proteins are attached to the RNA strand in such a way that one "side" of the strand is naked over its entire length. If this is true, then transcription would be possible without the necessity for displacement of the protein.

2. Structure of bacterial phosphoribosylpyrophosphate synthetase (Switzer).

The enzyme, isolated from Salmonella typhimurium, is not an easy enzyme to work with. It is poorly soluble in dilute solution and falls apart in the absence of Mg and ATP. Its molecular weight is about 180.000-210,000 and it falls apart into subunits of about 35,000 MW.

Our best estimate of the structure of the molecule is that of two triads Y-shaped with the branched ends overlapping so that the molecule very often presents the aspect of a playing card club. Our estimate of the subunit molecular weight (which involves a bit of game-playing) is in agreement with the physical data.

3. Structure of bacterial ethanolamine deaminase:(Babior)

Ethanolamine deaminase from Clostridium sp. is a coenzyme B_{12} requiring enzyme of 520,000 M.W. which has been shown to have two cobamide binding sites that are separate and independent.

The molecule consists of 10 or 12 subunits put together as a stack of two doughnuts each containing 5 or 6 subunits. We hope to solve the uncertainty of the subunit number very shortly. This structure would permit one cobamide binding site to be associated with each doughnut.

Significance to Cancer Research: A knowledge of the structure of macromolecules obtained by observations with the electron microscope provides in many cases an unequivocal basis for knowledge of their function and similar confirmation of physical-chemical data. Thus we have evidence that the RNA of vesicular stomatitis virus can be transcribed without removal of the associated protein.

Proposed Course of Research: To elucidate further the structure of macromolecules and correlate their structure with their function.

Honors and Awards: None

Publications:

Gessner, T.P., Himmelhoch, S.R., and Shelton, E.: Partial characterization of the protein components of eosinophil granules isolated from guinea pig exudates. Arch. Biochem. Biophys. 156: 383-389, 1973.

Stroud, R.M., Shin, H.S., and Shelton, E.: Complement components: Assays, purification and ultrastructural methods. In: Zacharia, T.P. (Ed.): Immune Response at the Cellular Level. Methods in Molecular Biology. Marcel Dekker, New York, 1973. Vol. VI.

Serial No. NCI-3213 SAME
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Carcinogenesis in vitro and its manifestations

Previous Serial No.: Same

Principal Investigator: K. K. Sanford

Other Investigators: S. L. Handleman, G. M. Jones, C. H. Fox,
T. P. Yang, M. L. Hursey, C. U. Garrison

Cooperating Units: Ram Parshad, Department of Pathology, Howard University
School of Medicine, Washington, D. C.;
Chou-Chik Ting, Laboratory of Cell Biology, NCI.

Man Years:		
	Total:	2-1/10
	Professional:	3/10
	Other:	1-4/5

Project Description:

Objectives:

1. To study factors influencing "spontaneous" and chemical carcinogenesis in vitro.
2. To identify any characteristics of cells in vitro that may be directly or indirectly associated with their neoplastic transformation.

Methods Employed: General methods will be described under each study.

Study 1. The influence of serum on "spontaneous" neoplastic transformation of cloned cell lines.

The type of serum has been shown to influence the spontaneous neoplastic transformation of mouse fetal cells in vitro; i.e., horse serum accelerates and fetal bovine serum delays this phenomenon when used as supplements to chemically defined medium. The following questions concerning mechanism were considered: (1) Did the two sera select from a heterogeneous cell population diverse cell types that differed in their susceptibility to spontaneous conversion? (2) Alternatively, could neoplastic transformation be produced within a single cell clone by transfer of some of the clonally derived cells to horse serum-supplemented medium? Horse serum was found to produce transformation in 3 cloned lines of mouse embryo cells, whereas during the same period of growth the cells in fetal bovine serum remained non-neoplastic by animal assay. This study has thus helped to answer the above questions and, in addition, has provided neoplastic and non-neoplastic cell lines of single cell origin for our comparative studies. These cell populations have been frozen in liquid nitrogen for subsequent use. A manuscript on this study is in preparation.

Study 2. Influence of hydrocortisone on cytotoxicity of certain polycyclic hydrocarbon carcinogens.

In our numerous efforts to obtain reproducible morphologic responses of hamster embryo cells to the chemical carcinogens, benzo(a)pyrene, 3-methylcholanthrene, or dibenzanthracene, we observed an influence of hydrocortisone (10^{-6}M) in reducing the cytotoxicity of these 3 carcinogens. Several possible mechanisms of action are currently being considered.

Study 3. Effect of oxygen and zinc on chromosome stability and neoplastic transformation of mouse embryo cells in vitro.

Analyses of sera showed that zinc levels in fetal bovine serum averaged 13x higher than in horse serum and that the concentration of zinc provided by 10% horse serum was exceedingly low. This study tested whether the increased chromosome instability and accelerated neoplastic transformation in horse serum-supplemented medium might result from a zinc deficiency. Dietary zinc has been reported to inhibit carcinogenesis in the hamster cheek pouch. Twelve cell lines were initiated from a pool of C3H mouse embryo cells. These were grown in chemically defined medium NCTC 135 + 10% horse serum supplemented with 0.1, 0.5, 5, or 10 uM Zn in the form of zinc sulfate. The lines on 0.5 uM Zn (fetal bovine serum equivalent) were gassed with either 1% O_2 or 20% O_2. The zinc concentration appeared to have no influence on chromosome stability, but the 1% O_2 as compared with 20% O_2 markedly increased chromosome stability. No effects of O_2 or Zn concentration on neoplastic transformation were observed. The $ZnSO_4$ levels did influence cell growth. A short manuscript on this study is being prepared.

Study 4. Plating efficiency in agarose in the differential diagnosis of neoplastic and non-neoplastic cells in culture.

Evaluation of this technique for discriminating between neoplastic and non-neoplastic cells is being carried out on the 5 pairs of cell lines that also are being assayed in vivo, examined for cytologic differences, differences in colony morphology, and susceptibility to activated macrophages (see 3 other projects on cytology, physiological manifestation, and spontaneous neoplastic change). To date,a consistent difference in plating efficiency in agarose of the neoplastic as compared with the non-neoplastic cells has been obtained in that the neoplastic cells produced many more colonies than the non-neoplastic which usually failed to grow. The mechanism of this effect is under study.

Study 5. Expression of fetal antigens in tissue cultures of fetal cells (collaboration with C. C. Ting).

The expression of fetal antigens in early and late passages of fetal tissue culture cells was investigated to determine: (1) whether fetal antigens can be retained in long-term fetal tissue culture cell lines; (2) the relationship between fetal antigen expression and neoplastic transformation; and (3) the possible usefulness of these cells as standard source of fetal antigens for immunodiagnosis or immunotherapy of tumors. Fetal antigens were found to be retained even after 5 years in culture lines derived from fetal tissues. In these cell lines no consistent qualitative or quantitative changes in fetal antigen expression could be correlated with neoplastic transformation. Since tumor cells and the fetal tissue culture cell lines shared some fetal antigens, the cell lines may be useful for immunodiagnosis or immunotherapy. This study has been completed and a manuscript submitted for publication.

Significance and Proposed Course:

The first study has helped to clarify the question as to whether the serum influence on neoplastic transformation results from selection of diverse cell types from a mixed population or whether transformation can be produced by horse serum in a clone. Also, neoplastic and non-neoplastic cell populations derived from a single cell without addition of viruses or chemical carcinogens are particularly valuable for comparative morphologic and biochemical studies.

The hydrocortisone effect is an interesting observation that will be pursued to understand possible mechanisms. The fact that growth of cells in horse serum-supplemented medium under a gaseous environment containing 1% rather than 20% O_2 (atmospheric) stabilizes chromosomes is an important observation that may provide a clue as to the mechanisms of chromosome breakage in vitro. This study will be pursued to analyze mechanism. Differential diagnosis of neoplastic cells in culture is important for two reasons: (1) Short in vitro assays will greatly facilitate experimental studies on carcinogenesis and factors influencing it; (2) Consistent differences between neoplastic and normal cells in culture may provide clues concerning the biochemical lesions in neoplastic change. This study will be actively pursued to meet the objectives.

Honors, Awards, and Other Activities:

1. Trustee of the Tissue Culture Association, Inc., and member of the Council
2. Member, Advisory Committee of the Animal Cell Culture Collection of the American Type Culture Collection
3. Member, Editorial Advisory Board of Cancer Research
4. Member, Terminology Committee, Tissue Culture Association, Inc.
5. Invitation to: Organize a Workshop on "Oncogenic Transformation in Culture" at the W. Alton Jones Cell Science Center, Lake Placid, N. Y.
6. Invitation to give two lectures for the Vertebrate Cell Culture Course at the W. Alton Jones Cell Science Center, Lake Placid, N. Y. and a lecture on "Nutritional Requirements of Cultured Cells" at the Institute of Medical Research, Camden, N. J.
7. Invitation to accept Honorary Degree of Doctor of Science, The Medical College of Pennsylvania and Hospital, Philadelphia, Pa.

Publications:

1. Sanford, K. K.: Cloning procedures; Animal Cells: Capillary Techniques. In Kruse, P. F., and Patterson, M. K. (eds.): Tissue Culture Methods and Applications. New York, N. Y., Academic Press, 1973, pp 237-242.
2. Sanford, K. K., Handleman, S. L., Burris, J. F., Fox, C. H., Mitchell, J. T., Jackson, J. L., Parshad, R., and Hursey, M. L.: Effects of chemical carcinogens on neoplastic transformation and morphology of cells in culture. J. Natl. Cancer Inst. (in press) 1974.

Serial No. NCI-3212
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on cell nutrition, growth, and neoplastic transformation in vitro

Previous Serial No.: Same

Principal Investigator: F. M. Price, K. K. Sanford

Other Investigators: R. R. Gantt, C. H. Fox, R. F. Camalier, S. L. Handleman

Cooperating Units: R. Parshad, Department of Pathology, Howard University, Washington, D. C.

Man Years:		
	Total:	2-1/5
	Professional:	1/5
	Other:	2

Project Description:

A program to determine the in vitro nutritional requirements of freshly isolated cells and long-term lines, both neoplastic and non-neoplastic, and to improve culture conditions so that rigidly controlled cells can be used to study the differences between non-neoplastic and neoplastic cells at nutritional, metabolic, karyologic and morphologic levels.

Objectives:

1. To develop a chemically defined medium which will allow more cell types including human cells to be grown for long-term in a protein-free milieu without experiencing the usual degenerative changes (senescence) characteristic of human cells in long-term culture.
2. To determine the influence of peptone on the growth and neoplastic transformation of cell lines derived from minced C3H mouse embryos.
3. To study the effect of serum fractions on the growth and neoplastic transformation of C3H mouse embryo cell lines.
4. To develop techniques for successful culturing of normal human epithelial cells. Once we are able to establish cell lines of normal human epithelial cells, we will transform these cells by chemical carcinogens and study differences between normal and neoplastic human epithelial cells at nutritional, metabolic, karyologic, and morphologic levels.

Methods Employed:

Freshly explanted mouse cells and long-term lines of mouse, hamster, monkey, and human cells were grown in media of varying formulation with or without protein supplement. Witte's peptone was used as a supplement to chemically defined media to determine its effect on growth and neoplastic transformation of freshly explanted C3H mouse cells. C3H mouse cells were also grown with large and small molecule fractions of mare and stallion serum to determine the effects of these fractions on growth and neoplastic transformation. Various techniques, to be described later, were carried out with normal human skin and intestine in order to develop methods for the successful cultivation of human epithelial cells.

Major Findings:

1. Development of a new chemically defined medium.

Attempts have been made to improve the fomulation of our culture medium in order to provide a medium which will support growth of many cell types without use of serum or protein hydrolysates. At the same time, we tried to eliminate unnecessary ingredients. A new formulation of medium, NCTC 163, has been prepared. It differs from NCTC 135 in several ways: (1) the buffering system has less bicarbonate and includes disodium phosphate to prevent an alkaline shift in atmospheric CO_2; (2) NCTC 163 has an increased concentration of glucose, several amino acids (particularly L-alanine and L-glutamine) and the B vitamins; (3) sodium pyruvate and putrescine are present in NCTC 163; and (4) 3 amino acids, 6 coenzymes, 3 fat-soluble vitamins, and 4 nucleic acid precursors are deleted from NCTC 163.

A study was carried out with 4 mammalian cell lines, one each of human, monkey, hamster, and mouse origin, to determine if these cells would grow as well in medium NCTC 163 as in medium NCTC 135. In a 7-day growth test, all but the monkey cells reached a higher population in NCTC 163 than in NCTC 135.

2. Growth of freshly explanted C3H mouse cells in medium supplemented with Witte's peptone.

Two cell lines from C3H mouse embryos were initiated in NCTC 163 supplemented with 15% Witte's peptone. The peptone supplement was about as effective as serum in supporting cell growth with the added advantage of being autoclavable. After several months in culture, cell lines in the peptone-supplemented medium underwent cytologic changes and neoplastic transformation as tested by implantation of cells in C3H mice.

3. The effect of serum fractions on the growth and neoplastic transformation of C3H mouse embryo cells.

Three pools of C3H mouse embryo cells were grown in NCTC 135 supplemented with large and small molecule fractions of fetal bovine serum, stallion serum, and mare serum. Best growth was obtained in medium containing the large molecule fraction of fetal bovine serum. In media containing both large and small molecule fractions of mare and stallion serum, growth was variable. Cells cultured in medium containing only the large molecule fraction of mare or stallion serum with one exception could not be established as long-term lines. Cells grown in the various fractions were examined at 3-week intervals for cytologic changes and also were injected periodically into syngeneic hosts for detection of neoplastic transformation. Analysis of these results is in progress. The results to date show a correlation between tumor production and morphologic changes. Cells grown in each of the 3 media containing the large molecule fraction of fetal bovine serum exhibited a markedly enhanced chromosome stability.

4. Development of techniques for the cultivation of freshly isolated human epithelial cells.

The source of tissue for this study has been skin taken from patients during surgery or from cadavers about 24 hours after death. Although human fibroblasts can be readily cultured, it is exceedingly difficult to cultivate human epithelial cells which appear to be more susceptible to chemical carcinogens than fibroblasts. First, the epidermis must be removed from the dermis. The only practical way to do this is by enzymatic dissociation of the two skin layers. This dissociation must be relatively rapid and yet the enzyme treatment must not damage the epithelial cells. We have found that enzymatic digestion of the dermis will occur more rapidly if a split thickness of skin is used. For this purpose, we have obtained a dermatone. As an example of this efficiency, we determined that the epidermis of a piece of whole thickness skin floated in 10 ml of 2.5% trypsin containing 4 ml versene (.02% w/v) could be removed from the dermis after 3 hours incubation at 37.5°C, as compared with 15 minutes for a split-thickness sample. We have also used viokase at percentages varying from 1 to 5% with about the same success as with the trypsin. Another method we have used for separation of the epidermis has been to soak the piece of skin overnight in a solution of medium containing 5-10% serum plus collagenase at a final concentration of 0.5 to 1.0 mg/ml. Apparently, however, some lots of collagenase are toxic to cells and each lot used must be tested for cytotoxicity before use. Another problem is that cells dissociated with collagenase attach very poorly. However, we have found that cells will attach to the flask surface if a minimum amount of medium is used and a sheet of perforated cellophane is laid over the cells.

We have also been able to initiate cultures from explants embedded in a plasma clot. Once the epithelial cells are planted, other problems develop. Fibroblasts because of their more rapid proliferation rate overgrow the epithelial cells. Furtunately, fibroblasts can be removed by trypsin while epithelial cells are resistant. Therefore, we have found it necessary to trypsinize once or twice a week to remove fibroblasts. We are still experimenting with various media formulations and protein supplements to obtain optimal conditions for cell growth. Thus far, it appears that horse serum is a better supplement than fetal bovine serum.

Significance and Proposed Course:

1. A new and improved chemically defined medium, NCTC 163, has been developed for the growth of long-term cell lines of diverse species. This medium, in the absence of added serum or proteins, gives a higher yield of cells than NCTC 135. This study has been completed and a manuscript submitted for publication in In Vitro.

2. When supplemented with Witte's peptone, this medium formulation supports the growth of freshly explanted mouse cells, so that it is now possible to initiate cells in a chemically defined medium with an autoclavable supplement eliminating possible contamination with viruses or mycoplasma.

3. Analysis of data regarding the large and small molecule fractions of fetal bovine, mare, and stallion serum is being made now. These data may give information on factors causing spontaneous neoplastic transformation.

4. We will continue to improve methods for establishing and cultivating human epithelial cells. Once we are proficient in growing these cells, we will attempt to induce neoplastic transformation with chemical carcinogens and study the differences between normal and neoplastic human epithelial cells with regard to morphology, karyology, metabolism, and nutrition.

Serial No. NCI-5228
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Cytology of Neoplastic Transformation In Vitro

Principal Investigators: S. Handleman, K. K. Sanford

Other Investigators: C. H. Fox, R. W. Tucker, F. M. Price, G. M. Jones, R. F. Camalier, C. U. Garrison

Cooperating Units: None

Man Years:	Total:	1-3/5
	Professional:	3/10
	Other:	1-3/10

Project Description:

Objective: To identify any cytologic characteristics of cells in culture that may be directly or indirectly associated with their neoplastic transformation in vitro and then to utilize those cytologic characteristics for studying the induction of neoplastic transformation by chemical carcinogens, as well as for following the progressive, often very subtle changes associated with the spontaneous neoplastic transformation of cells in vitro.

Methods Employed: A combination of cytologic criteria previously described and validated for C3H mouse cell cultures is used to study individual cells on a 24-48 hour cover-slip preparation. Colony morphology is used to analyze cell populations on 10-13 day plastic petri dish or T25 flask preparations.

Cytologic Studies on Cells Treated with Chemical Carcinogens: This study entitled "Effects of Chemical Carcinogens on Neoplastic Transformation and Cytology of Cells in Culture" was outlined in last year's annual report. It has been completed and a manuscript is in press.

A random criss-cross growth pattern of spindle-shaped cells has been considered a "marker" of carcinogen-induced "transformation." In the present study criss-cross growth pattern was observed soon after carcinogen treatment in some cultures but these cultures did not give rise to sarcomas when injected. Most of the cultures that ultimately grew as sarcomas when injected did not exhibit the criss-cross growth pattern. It appeared that this particular marker could not be relied upon as the only diagnostic feature of carcinogen-induced neoplastic transformation.

Although no single morphologic event could be definitely associated with neoplastic transformation, combinations of the following morphologic features showed a high correlation with tumor production: increased nuclear:cytoplasmic ratio; cording; spindling or rounding of cells; criss-cross growth pattern; clumping; cell separation; heterogeneity in cell size and shape; cannibalism; heterogeneity in nuclear size and shape; nuclear abnormalities including multinucleation, nuclear budding, nuclear bridges, abnormal mitoses; and heterogeneity in nucleolar shape, size, and number.

Cytologic Studies on "Spontaneously" Transformed Cells:

Study 1: The cytology of cells undergoing neoplastic transformation in culture.

The objective is to study the progressive cytologic changes of embryo (fetal) cells of human, rat, hamster, and mouse origin during long-term culture, and to establish more quantitative, objective cytologic criteria for neoplastic transformation of cells in vitro and a systematic approach to in vitro diagnosis of neoplastic state. Minor objectives are: 1) to attempt to apply criteria established and validated for rodent cells to human cells; 2) to compare responses of cells in a new chemically defined medium with horse serum or fetal bovine serum supplement with earlier results with serum-supplemented medium NCTC 135; 3) to determine whether human cells can be established as permanent long-term lines in the new medium formulation.

The following pools of cells were used to initiate cell lines: 3 pools of 13-day C3Hf mouse embryo cells, 3 pools of 18-day Fisher rat embryo cells, 3 pools of 14-day Syrian hamster embryo cells, and 2 pools of human embryo cells. The derivative lines were grown on an improved MEM formulation, supplemented with either horse serum or fetal bovine serum. A permanent record of cytologic characteristics in the form of stained coverslips has been prepared. The rodent cells have been assayed in vivo for neoplastic transformation. The human cells have been examined for changes in enzyme isozymes.

In results to date all the human lines exhibited a finite life time in culture and none showed cytologic evidence of neoplastic conversion. Neoplastic conversion, as determined by in vivo assay, occurred in 4 mouse lines, 4 rat lines, and 1 hamster line. These results correlated well with the results of cytologic diagnosis.

Further analysis of the cytologic preparations is required before definite conclusions can be made concerning the following: the applicability of certain in vivo cytologic criteria to cells in culture; the feasibility of a systematic key to cytologic characteristics used in predicting and diagnosing neoplastic transformation in vitro.

Study 2: The use of colony morphology for the diagnosis of neoplastic cells in culture.

The objective is to use colony morphology to analyze cell populations and to give some estimate of what percentage of neoplastic cells are found in a cell population. Suspensions of single cells are plated in plastic petri dishes or T25 flasks at low inoculum sizes (usually 200-1000 cells per vessel) and are allowed to grow undisturbed for 10-13 days. These preparations are then fixed and stained and examined for colony morphology. This method presumes that when a single cell suspension is plated out, each attached cell will produce a colony. The percentage of neoplastic cells in the total cell population can be determined based on the kinds of colonies neoplastic cells will produce.

This study is designed to bring together and correlate several types of in vitro assays with the ultimately conclusive in vivo assay. Paired cell lines, i.e., neoplastic and non-neoplastic cell lines from the same embryo pool or from one cell, are grown up in vast quantities and injected at various inocula into their appropriate hosts. A portion of the cell suspension is set aside and used for these different assays:

1. The 24-48 cover-slip preparation used to study individual cells cytologically.
2. The plated petri dishes and T25 flasks used to study colony morphology and to analyze the cell populations.
3. The colony-forming ability of cells in agarose used to differentially distinguish non-neoplastic from neoplastic cells.
4. The activated macrophage assay used to differentially distinguish non-neoplastic from neoplastic cells.

To date, few of the cytology or morphology preparations have been completely analyzed. Additional data from the injections are also needed to: 1) assess the value of these in vitro assays and their correlation to the in vivo assay, and 2) determine how well population analysis is correlated to latent period in vivo.

Serial No. NCI-3264
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Spontaneous neoplastic change during growth in culture

Principal Investigator: R. W. Tucker

Other Investigators: K. K. Sanford, G. M. Jones, S. L. Handleman

Cooperating Units: M. S. Meltzer, Biology Branch, NCI
A. C. Breuer, Behavioral Biology Branch, NICHHD

Man Years:		
	Total:	2-3/10
	Professional:	1
	Other:	1-3/10

Project Description:

Objectives:

To study some of the physical and behavioral changes that cells undergo during spontaneous neoplastic change and/or long-term culture in vitro. Four objectives are the following:

1. To establish whether cells adapted to serum-free chemically defined medium are producing growth-stimulating substances required by the serum-dependent progenitor cells.
2. To assess the usefulness of the tumor-specific cytotoxicity of activated macrophages for discriminating between neoplastic and non-neoplastic cells in culture.
3. To study the differences in DNA repair capability in cell lines before and after neoplastic change.
4. To study microtubular arrays in normal and neoplastic cells.

Methods Employed:

1. Growth stimulatory substances: The media conditioned by serum independent cells is assayed for growth-stimulatory substances able to sustain growth of serum-dependent cells.
2. Cytotoxic interactions between activated mouse peritoneal macrophages and tissue culture cells will be evaluated as indicators of neoplastic change. BCG will be used for activation.
3. DNA repair will be studied by conventional autoradiographs of UV irradiated cells grown in tritiated thymidine.
4. Microtubular arrays will be measured by quantitative polarization microscopy of the birefringence of the mitotic spindle and pseudopod fibrillar material in both normal and neoplastic cells.

Major Findings:

1. Serum-independent cells do not release appreciable amounts of serum-like substances into the surrounding medium which could then support the growth of their serum-dependent progenitor cell populations.

2. In five neoplastic, non-neoplastic cell pairs, the neoplastic cell line is more susceptible to BCG-activated macrophage than are the non-neoplastic cells. This reaction is a quantitative dose response, crosses species lines, and occurs even with the progeny of a single cloned cell. A film of BCG-activated macrophages has shown definite cytostaxis and cytostatic activity against neoplastic cells. A comparison between in vivo tumor-latency periods and in vitro degree of reaction to macrophages showed no correlation among the different neoplastic cell lines.

3. DNA repair in rodents following UV irradiation can be measured and appears to be quantitatively less (fewer grains/nucleus) than human cell lines.

4. Polarization optics of cells growing in perfusion chamber have shown these areas of birefringence: 1) perinuclear, 2) pseudopod, 3) cell-to-cell contact.

Significance and Proposed Course:

1. The fact that cells adapted to serum-free conditions do not release substance(s) into the medium which will support the growth of their serum-dependent progenitor cells implies that serum-free adaptation requires the acquisition of different serum requirements or the means of satisfying the same serum requirements by macromolecules not released into the medium. Here the derivation of serum-independent lines directly from the serum-dependent line and the use of identical growth medium allows us to interpret our results unambiguously. There is no contribution by mixing different media or different cells together. Ultimately this kind of cell pair will yield more information concerning the role serum plays in normal growth, and in the decreased serum requirement of most neoplastic cells.

2. BCG-activated peritoneal macrophages can distinguish between neoplastic and non-neoplastic cells. Such a quantitative dose response reaction can be scored and the scores tallied for different cell lines. Certainly, it is significant that the macrophage reaction can distinguish between neoplastic and non-neoplastic regardless of species, time in culture, or homogeneity of initial pooled cells producing the pair. But, more interestingly, it may be possible to establish enough score-cell pair correlations that it would be possible from the score of a given cell line to establish whether that cell line was neoplastic or non-neoplastic. Further studies will also be done on mixtures of neoplastic and non-neoplastic to establish if the differences of susceptibilities still hold, or if the non-neoplastic cells will also die as an innocent bystander reaction.

Films will be done in order to evaluate whether macrophages are attracted to target cells by analyzing the paths of the macrophage in the vicinity of a moving target cell.

3. DNA repair measured by autoradiography is able to detect extensive unscheduled DNA synthesis following UV irradiation in both rodent and human cell lines. A continuous treated thymidine label shows a complete cessation of cells entering or leaving S (DNA synthesis). The accompanying lightly labeled nuclei that persist throughout the continuous label are therefore interpreted to represent DNA repair incorporation of tritiated thymidine. This experimental system can, therefore, be used to follow changes in DNA repair processes during the process of neoplastic conversion in vitro.

4. Polarization microscope and perfusion chamber have been used to demonstrate birefringent fibrillar arrays in living mammalian cells under high resolution optics in 3 regions of the cell: 1) perinuclear, 2) cell-to-cell contact, 3) central areas of pseudopod extension. The measurement of total retardation of these fibrillar arrays is 1-2 nm, with a (+) sign of birefringence. These data indicate that the birefringent structures may be composed of packed longitudinal fibrils, as in the mitotic spindle of these same cells.

Serial No. NCI-3234A
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Primary biochemical alterations leading to neoplastic conversion.

Previous Serial No.: Same

Principal Investigator: R. R. Gantt

Other Investigators: K. K. Sanford, T. P. Yang

Cooperating Units: G. H. Smith, Laboratory of Biology, NCI

Man Years:		
	Total	3
	Professional	1
	Other	2

Project Description: To elucidate the primary chemical alterations which are necessary biochemical changes leading to neoplastic transformation and to devise methods to detect and control the transformation based on these chemical alterations.

Objectives: To identify primary changes in the methylation of cellular and viral nucleic acids during neoplastic transformation and to exploit these changes to develop an assay for, and possible control of, neoplastic transformation in vitro.

Methods Employed:

1. In order to identify primary changes in the methylation of nucleic acids, significant differences are first being established in paired cell lines before and after neoplastic conversion. The differences will then be correlated with conversion time and with the method of conversion (i.e., viruses, chemical carcinogens, or the horse serum-fetal bovine serum system). These differences are monitored by double isotope co-elution chromatography and enzyme analysis.

2. The discovery of N^2-guanine RNA methyltransferase in AMV has suggested that other oncogenic RNA viruses may have the same or similar activities associated with the virion. Therefore, available viruses have been examined for RNA methylase activities, and virus-infected and uninfected chick fibroblasts are being compared for methylase differences.

Major Findings:

1. The C-type sarcoma viruses have two methylase activities (N^2-methylguanine and 1-methyladenine), while the leukemogenic viruses have only one activity. (Gantt, Smith and Julian, Virology 52:584, 1973).

2. In contrast to the C-type RNA tumor viruses (AMV, RSV, RAV-1, MSV, MLV), cytoplasmic A particles have N^2-guanine methylase activity which is not surfactant-dependent and the activity remains with the particle after surfactant treatment. (Abstract presented at the VIIIth International Meeting on Mammary Cancer in Experimental Animals and Man, 1973).

3. Developed a rapid chromatographic assay for RNA methylases (Gantt and Julian, Anal. Biochem. 54:283, 1973), and recently a pressure, high resolution chromatographic assay (Gantt, in preparation).

4. Demonstrated that 5-methylcytosine is equally distributed on both strands of mouse satellite DNA. (Gantt, Montes de Oca, Evans, In Vitro 8:288, 1973).

5. Found in mammalian cells a DNA methylase activity, 5-methylcytosine, associated with a nuclear membrane fraction which contains DNA polymerase activity. In addition, a second activity, 1-guanine methyltransferase, was found which has not been seen before. (Longfellow, Gantt, Smith, in preparation).

6. In the mouse mammary tumor system no correlation exists between RNA methylase capacities of primary tumor tissue extracts and presence of the etiologic agents MTV, NIV, MTV and NIV together, or "spontaneous" tumors. Further, normal lactating mammary tissue has a greater methylating capacity than any mammary tumor tissue assayed to date; this result, where normal tissue extracts have capacities greater than malignant tissue extracts, is generally not the case.

Significance:

The studies to date on the RNA methylases still corroborate the "aberrant methylation" hypothesis of malignant conversion, where "aberrant methylation" is taken to mean "different" rather than the generally observed "greater methylation" capacity of malignant tissue. The result that RNA oncogenic viruses are associated with a specific RNA methylase provides a focal point for further work. Insight into the functions of RNA methylation may be gained if the methylase associated with the C-type viruses is found to be important for the virus life cycle.

The DNA methylation studies show that the methyl group heterogeneity associated with the DNA fraction in early comparisons of paired cell lines is probably due to RNA not DNA. The RNA is apparently associated with the DNA but not covalently bound to the DNA. This finding suggests that other RNA fractions besides tRNA may be involved in "aberrant methylation."

Proposed Course of Investigation:

1. Establish whether the N^2-guanine methylase is essential for the virus life cycle.
2. Identify the differences in the paired cell lines using the newly developed methods and establish whether they are general.
3. Determine whether the mammalian DNA requires methylation to serve as a template for replication.
4. Determine whether N'-methylguanine is present in the DNA of intact mammalian cells and if it is associated with the cell cycle.

Publications:

1. Stromberg, K. J., Gantt, R. R., and Wilson, S. H.: Structural studies on avian myeloblastosis virus: Conditions for isolation and biochemical characterization of the core component. Biochim. Biophys. Acta 304: 1-11, 1973.

2. Gantt, R. R., Smith, G. H., and Julian, B. T.: Base specific methylase activity in RNA tumor viruses: Avian leukosis virion-associated RNA methylase(s). Virology 52:584-586, 1973.

3. Gantt, R. R., and Julian, B. T.: A rapid column chromatographic procedure for the assay of RNA methyltransferases. Analyt. Biochem. 54: 283-285, 1973.

4. Gantt, R. R., Montes de Oca, F., and Evans, V. J.: Methylation of satellite DNA in mouse neoplastic and nonneoplastic cell cultures. In Vitro 8:288-294, 1973.

5. Gantt, R. R.: Are nucleic acid modifications involved in neoplastic transformation? J. Natl. Cancer Inst., in press, 1974.

6. Longfellow, D. G., Gantt, R. R., and Smith, G. H.: Isolation of a membrane-deoxyribonucleoprotein complex from mouse cells in S-phase, containing DNA polymerase and DNA methylase activities. To be submitted.

7. Gantt, R. R., Smith, G. H., and Julian, B. T.: Comparison of RNA methylases in normal mammary tissue, MTV-induced, NIV-induced, and "spontaneous" mouse mammary tumors. In preparation.

Serial No. NCI-3257
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Factors influencing the growth and neoplastic conversion of mammalian cells in vitro

Previous Serial No. Same

Principal Investigator: W. G. Taylor

Other Investigators: K. K. Sanford, R. R. Gantt, F. M. Price, A. Kerr, R. F. Camalier, T. P. Yang

Cooperating Units: V. J. Evans, American Foundation for Biomedical Research, Rockville, Maryland
R. Parshad, Department of Pathology, Howard University School of Medicine, Washington, D. C.

Man Years:	Total:	2-3/4
	Professional:	3/4
	Other:	2

Project Description:

1. Effect of inoculum size on development of malignancy in rapidly subcultured cloned cell lines.
2. Effects of gaseous oxygen on cell growth.
3. Further studies on the rapid adaptation of mammalian cells to growth in chemically defined medium.
4. Further biochemical analysis of peptone dialysate.

Objective No. 1:

Density-dependent inhibition of mitosis (or "contact inhibition" of mitosis) is thought to distinguish non-neoplastic from neoplastic cells grown in tissue culture. According to this hypothesis, once a confluent monolayer of non-neoplastic cells has formed, the rate of cell division drops considerably; in contradistinction, neoplastic cells continue to divide and form multiple cell layers. In support of this hypothesis, Aaronson and Todaro (Science 162:1024, 1968) carried one line of BALB/c embryo cells at two different inoculum sizes ($3x10^5$ and $12x10^5$/50 mm petri dish, i.e., BALB/3T3 and BALB/3T12, respectively) on a rigid culture transfer schedule. Cells carried at the higher inoculum size underwent neoplastic transformation as evidenced by production of tumors in irradiated syngeneic hosts; cells subcultured at the lower inoculum size failed to become tumorigenic. These observations were interpreted as follows: "lines that grow efficiently in the presence of extensive cell-cell contact readily produce tumors; those lines where cell division is arrested by neighboring cells in tissue culture are also unable to grow in the animal."

Our objectives were as follows: (1) to establish with two single-cell clones whether this phenomenon (i.e., enhanced neoplastic transformation by serial propagation of cells at high as compared with low inoculum size) is reproducible; (2) to assess the relationship, if any, between density-dependent inhibition of growth and neoplastic or non-neoplastic state.

Methods Used.--Cell strains NCTC 7914 and 7915 were derived from single cell clones of a low passage C3H/f mouse embryo cell culture. Parent cultures at the 12th-13th passage level were each divided into two experimental sets; one set of cultures is routinely subcultured at 10^5 cells/T15 and becomes confluent in about 4 days, while the other set is subcultured at 4×10^5 cells/T15 and becomes confluent at about 48 hours. Each set of cultures is carried under a rigid transfer schedule. Neoplastic transformation of the cultures is assessed by: intraocular injection (Evans, et al., J. Natl. Cancer Inst. 32:89, 1962), morphologic criteria, and growth in agarose suspension culture. Growth rate and plating efficiency are also determined.

Major Findings.--(1) To date, no tumors have been observed in mice inoculated with cells carried at the low inoculum size, whereas tumors have developed from cells carried at the high inoculum size. Injections are being continued to obtain statistically significant data. (2) Based on biweekly cell counts, no marked difference in growth rate has been observed.

Significance and Proposed Course.--It is important to know whether the loss of density-dependent inhibition of growth is associated with the acquisition of neoplastic transformation; more precise information will be obtained with populations of fibroblast-like cells derived from a single cell. Also, any information on methods for controlling or preventing spontaneous neoplastic transformation is essential for further use of cell lines for experimental or clinical purposes. In vivo assays will be completed and growth curves of each population will be obtained to determine saturation densities. Other assays (e.g., for endogenous viruses, density-dependent inhibition of growth) will be performed in an effort to analyze the mechanism of transformation.

Objective No. 2.: To ascertain the growth regulatory effect of oxygen on mammalian cells in culture.

Methods Used.--Analytically prepared mixtures of high purity gases have been used in the gas phase for clonal growth or mass cultures. Atmospheric (20%) and low concentration (1%) O_2 were compared. In clonal growth studies, colony counts were used as an end point, and in mass culture studies cell numbers were determined.

Major Findings.--(1) Equal or better plating efficiency (i.e., colony development) was observed in cultures of rodent, monkey, or human cells when grown under a gas phase containing 1% O_2 as compared with atmospheric (20%).
(2) The reducing agents, cysteine or thioglycollate at the broad range of concentrations tested did not alleviate the growth inhibition observed at

20% O_2. This effect was independent of the type of medium, source of serum, or use of a feeder layer. (3) Human diploid fibroblasts exhibit a marked sensitivity to atmospheric O_2 especially at alkaline pH, whereas at low O_2 and physiologic pH, high plating efficiencies were obtained. Heteroploid monkey cells showed a reduced sensitivity to atmospheric O_2 and clones developed even at alkaline pH. (4) In studies to date, low O_2 does not enhance growth of mass cultures of human diploid fibroblasts. A combination of low O_2 and use of collagen substrate promotes better growth of high passage (phase III) human fibroblasts but does not prevent their ultimate degeneration.

Significance.--In current plating methods cells are grown under atmospheric O_2 (18-20%) whether deliberately introduced as 10% CO_2 in air or present as the residual gas in the culture vessel. Under these conditions 50-75% of inoculated cells frequently fail to survive and an even higher proportion are lost if cells are from primary or low-passage culture. The present studies on fetal rodent and human, and adult monkey cell cultures suggest that enhanced colony development under "low O_2" conditions is a general phenomenon applicable to all cultured cells regardless of species and whether neoplastic or non-neoplastic. Possibly freshly explanted or normal diploid cells are sensitive to a gas phase of atmospheric O_2, while long-term heteroploid cultures have "adapted" with time and are less susceptible. An alternative interpretation is that fetal cells exhibit greater O_2 sensitivity than those of adult origin.

These observations on cells in culture are of interest because oxygen tensions in many normal tissues in vivo are reputedly considerably less than atmospheric (approaching 3-5% O_2). Separate studies suggest that atmospheric O_2 promotes karyotypic instability and thus may be a factor in the evolution of long-term heteroploid cell lines (see NCI-3213). Improving the plating efficiency of freshly isolated, non-neoplastic cells is essential to studies on chemical and spontaneous transformation.

Proposed Course.--Studies planned or in progress are: (1) Assessment of the O_2 sensitivity of low-passage adult human fibroblasts to determine whether this phenomenon is limited to cells of fetal origin; (2) Evaluation of the effect of O_2 concentration on mass culture to determine the relationship between cell density and O_2 sensitivity in stationary, rocked, and roller tube cultures; (3) Studies to explore the hypothesis that for optimal growth the concentration of O_2 varies directly with cell number.

Objective No. 3.: The aim is to devise or improve culture media and techniques and thereby promote the rapid "adaptation" of established mammalian cell lines to growth in serum-free, chemically defined medium (CDM).

Methods Used.--Non-neoplastic and neoplastic cell lines have been used. Medium NCTC 135 is supplemented with decreasing amounts of fetal bovine serum (FBS) in combination with whole peptone or peptone dialysate (PD).

Major Findings.--(1) Decreasing serum concentrations concomitant with the use of peptones or peptone dialysate (PD) promotes the rapid adaptation of cells routinely grown in medium NCTC 135 plus 10% v/v FBS to continuous growth in serum-free, chemically defined medium. With a combined serum-peptone supplement, this adaptation required 160 days versus over one year by other methods. (2) This culture regime caused no frank changes in chromosome numbers or type. (3) Greater cell numbers were observed if PD were used in place of the whole peptone. In one case the degeneration of a cell line grown in CDM for more than 20 weeks was immediately reversed by PD. (4) At the concentrations tested, protective agents or plasma extenders such as methylcellulose, polyvinylpyrrolidone and dextrans have failed to supplant the macromolecular serum requirements for mass culture of neoplastic cells.

Significance and Proposed Course.--One reputed metabolic difference between normal and neoplastic cells is the reduced serum-dependence of neoplastic cultures. Selective procedures for discriminating between normal and neoplastic cells, both in mass culture and under conditions of clonal growth, are needed. These studies provide baseline data needed for devising such selective media.

The culture regime represents an easy, repeatable method for adapting available cell lines to growth in serum-free medium. Elimination of serum reduces the chance of adventitious microbial contamination, eliminates heterologous serum proteins, etc., and provides serum-free medium in which to prepare viral antigen, cell products, etc. It is hoped these techniques can be successfully applied to primary human cell culture.

Objective No. 4.: The immediate objective is the characterization of the component(s) in peptones which promote continued survival and growth in the absence of serum. The long-term objective is the development of (1) heat-stable serum-substitutes for primary or diploid human cells, and (2) better chemically defined media.

Methods Used.--Dialysis of whole peptones effects a 100-fold increase in specific activity as measured by cell growth. Analysis of peptone dialysate (low molecular weight mixture) for the presence of specific components is followed by preparation of experimental media with detected (or suspected) metabolites. A variety of cells which require peptone or peptone fractions for continued growth are available for the bioassay of test media.

Major Findings.--(1) PD contains no detectable reducing sugars (Molisch test) though a final glucose concentration of 200 mg% enhances peptone-dependent cell growth. Tricarboxylic acid cycle intermediates are not necessarily detected by the Molisch method. In the absence of PD or with only 0.5 mg PD/ml (20% of that normally used), sodium pyruvate and oxaloacetic acid markedly enhanced survival and short-term growth of RH-PD (rabbit heart); a-ketoglutaric acid, succinic and fumaric acids were less beneficial. Lactic acid and other TCA intermediates failed to promote any growth. None promoted growth to the same degree as PD-supplemented control cultures. (2) Ongoing studies with putrescine suggest it may enhance survival and growth of RH-PD cells. (3) Choline (at 2-20 ug/ml medium), several glycine peptides, zinc, and iron failed to enhance survival and growth of peptone-dependent cells, as determined by quantitative growth studies.

Significance and Proposed Course.--(1) The data from studies with TCA intermediates is particularly useful in planning future studies on the effect of low oxygen (1%) on clonal growth of mammalian cells. (See project #2, this report). (2) These studies eliminated a large group of metabolites reported to be essential to cell growth by other investigators. Ongoing work includes studies to obtain chemical proof of the peptide nature of this growth factor(s), and further separation and purification by chromatographic means so that better synthetic media can be designed.

Publications:

1. Taylor, W.G., Sanford, K.K., Richter, A., Hursey, M.L., and Evans, V.J.: Effect of reducing agents and oxygen on plating efficiency of some mammalian cells in culture. J. Natl. Cancer Inst. 52:763-767, 1974

2. Taylor, W.G., Richter, A., Evans, V.J., and Sanford, K.K.: Influence of oxygen and pH on plating efficiency and colony development of WI-38 and Vero cells. Exp. Cell Res. (in press).

3. Taylor, W.G., Evans, V.J., and Pumper, R.W.: Studies on a serum substitute for mammalian cells in culture. II. Analyses of fractionated peptone dialysate and the use of glycine peptides. In Vitro 9:278-285, 1974.

4. Taylor, W.G., and Evans, V.J.: Preparation and use of replicate mammalian cell cultures. In Prescott, D. (ed.) Methods in Cell Biology, Vol. 8, New York, N. Y., Academic Press, Inc., 1974, pp. 47-73.

5. Taylor, M.J., Kohn, B.D., Taylor, W.G., and Kohn, P.: Studies on nucleotide analogues. I. Synthesis of two 9-a-D-mannofuranosyladenine phosphates and their inhibition of adenylate kinase. Carbohydrate Res. 30:133-142, 1973.

Serial No. NCI-3262
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The development of a homeostatic cell culture system and/or growth promoting system for cells in vitro

Previous Serial No.: Same

Principal Investigator: W. G. Taylor

Other Investigators: K. K. Sanford, R. R. Gantt

Cooperating Units: V. J. Evans, V. P. Perry, C. Kroener,
American Foundation for Biological Research,
Rockville, Maryland

Man Years: Total: 1/8
Professional: 1/8
Other: 0

Project Description:

Objective: The immediate objective is the development of a perfusion culture system in which in vitro modulations (nutritional variation, changes in physico-chemical parameters) can be measured and controlled within specific limits. The long-term objectives include the use of this system to study the events preceding in vitro neoplastic conversion.

Major Findings: The automatic monitoring of pH, pCO_2, pO_2, redox potential, and flow rate has been successfully miniaturized. A variety of technical problems have impeded progress, but it is hoped assessment of the system's capability will be resumed in the near future.

Significance: The pulsatile perfusion system is designed to pump culture medium through cell, tissue, or organ cultures while maintaining a predetermined systolic-diastolic pressure. Instrumentation continuously measures pH, pCO_2, and pO_2. Glucose determinations, amino acid utilization, etc., are planned. Once "physiologic" limits are established, automatic maintenance of these parameters within specific limits will provide a "homeostatic" culture system with which to monitor and control factors that influence neoplastic conversion in vitro.

This system should provide an ideal milieu in which to grow lymphocytoblasts, prepare mass amounts of cells (see also NCI-3263), hard-to-obtain natural products such as certain hormones, etc.

Proposed Course:

Specific studies include:

1. Assessment of different dissolved O_2 concentrations and the effect of systolic-diastolic pressure on (a) growth of primary cell cultures, (b) in vitro chromosome stability, and (c) spontaneous neoplastic transformation.
2. Analysis of metabolic requirements of human cell cultures, particularly for specific cell types that are difficult to propagate in vitro.

Publications:

Perry, V. P., Evans, V. J., Kroener, C. A., and Taylor, W. G.: A continuous pulsatile perfusion apparatus for mammalian cells in culture. (In preparation).

Serial No. NCI-3263
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Developmental research on new methods for the cultivation of very large numbers of human and other mammalian cells in vitro

Previous Serial No.: Same

Principal Investigator: W. G. Taylor, C. H. Fox, R. F. Camalier

Other Investigators: K. K. Sanford, F. M. Price

Cooperating Units: V. J. Evans, C. A. Kroener, American Foundation for Biological Research, Rockville, Maryland

Man Years:		
	Total:	1-1/5
	Professional:	3/10
	Other:	9/10

Project Description: Non-neoplastic and some neoplastic cells require a surface upon which they can attach, migrate, and divide. This study has evaluated a new means of growing gram amounts of "substrate-dependent" mammalian cells in a single culture vessel.

Objectives: The immediate aim was to determine those conditions essential to the growth and recovery of gram amounts of mammalian cells in the new, commercially available Sterilin Bottle^R.

Methods Used: The Sterilin Bottle^R is a cylindrical culture vessel which houses a long, rectangular plastic film. The film is rolled into an Archimedes' spiral and the layers of plastic spaced by thin strips of corrugated plastic. Cells and medium (1.6 - 1.7l) are introduced through ports on top of the vessel, and the culture medium aerated and gently circulated by intermittent gassing with 5% CO_2 in air. Since the inner layers of plastic cannot be visualized, cell growth is assessed indirectly through measurements of available glucose calculation of glucose utilization rates.

Major Findings:

1. Strain L (NCTC 2071) cells in fully chemically defined medium NCTC 135, and monkey kidney cells (MK_2) grown in semi-defined peptone-supplemented medium will attach and grow in this bottle (this is variable in plastic culture flasks).
2. Depletion of available glucose (and probably other nutrients) occurs rapidly, and complete replacement of the medium with fresh fluid is essential for successful use.
3. Gram quantities of cells can be grown in this vessel, but at the present time must be mechanically removed from the growth surface.

Significance and Proposed Course:

The usefulness of the Sterilin Bottle^R and related culture devices is obvious. This vessel permits production of large populations of substrate-requiring mammalian cells for tumorigenesis assays, biochemical analysis of metabolites elaborated into the growth medium, membrane or intracellular components (membrane antigens, mitochondria, nuclei, etc.), and potentially may be useful in the production of vaccines, provided the biohazard aspects can be controlled.

The proposed course of study includes: 1. Further attempts to develop a rapid, physiologic means of dispersing the cell population from the growth surface; 2. analysis of the growth medium to provide base line data on the metabolism of mammalian cells--and particularly human cells and primary cultures--under the growth conditions imposed by the geometry of the culture vessel and the mass of cells attainable; 3. the development of improved culture medium for specific use in culture vessels of this type; and 4. the use of this and similar vessels to explore the effects of variations in physiologic parameters on neoplastic transformation, both "spontaneous" and chemically induced.

A manuscript is in preparation.

Serial No. NCI-5229
1. Laboratory of Biochemistry
2. Cell Physiology & Oncogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physiological manifestations of neoplastic transformation in vitro

Principal Investigator: C. H. Fox

Other Investigators: K. K. Sanford, S. L. Handleman, G. M. Jones, F. M. Price

Cooperating Units: J. A. Dvorak, NIAID, Laboratory of Parasitic Diseases
O. W. McBride, NCI, Protein Chemistry Section, LB
M. H. Cottler-Fox, NCI, Viral Oncology Branch
B. K. Wetzel, NCI, Dermatology Branch

Man Years:	Total:	7/10
	Professional:	3/5
	Other:	1/10

Project Description:

Objective: To isolate those parameters that accompany neoplastic transformation, and using conventional chemical and physical methods to attempt to elucidate either the neoplastic transformation process, or to develop techniques for the detection of transforming influences in cell cultures.

Methods Employed: The methods to be employed will be described under each study heading.

Study 1. Comparative morphology of paired lines of neoplastic and non-neoplastic cells. This study concerns the development of criteria of neoplasia for cultured cells. These studies are being conducted by several different methods. Cells isolated from tissues of several species of rodent are cultivated until they become neoplastic as judged by the formation of tumors upon injection into a suitable recipient. There are two aspects to this type of study. First, the way in which the cells grow in colonies is studied by fixing and staining cultures and observing the colonies (sometimes called clones) for characteristic "transformed" morphology. A second aspect involves the structure and properties of the cells themselves. In this study we shall prepare cells from paired lines of neoplastic-non-neoplastic cells each pair derived from a single cell. These will be studied by the use of modern light microscopic techniques such as Smith differential interference, Mach-Zehnder transmitted light interference, and Nomarski interference. This will allow us to make quantitative comparisons of such parameters as nuclear:cytoplasmic ratio, dry mass of cells, mean cell thickness, and cell and nuclear volume.

Another aspect of this work is a study in progress concerning the comparison of neoplastic and non-neoplastic cell lines using the combination of timelapse cinephotomicrography and scanning electron microscopy. In this study cells are grown on cover slips under the cinephotomicrographic camera and after some time are fixed in glutaraldehyde and dried by the critical point method, whereupon they are examined in the scanning electron microscope. This study is in collaboration with Bruce Wetzel of Dermatology, NCI. It allows the comparison of cells of known genealogy with respect to numbers of microvilli and surface peculiarities.

We intend to use the film produced in the project described above for a further study of the relative rates of motion of cells in culture, their projected surface area, and the relative nuclear sizes of neoplastic and normal cells of common single cell origin.

Study 2. Metabolic products of neoplastic and non-neoplastic cells. This study, now in progress, concerns a search for products of metabolism of both normal cells and neoplastic cells. The search now centers around the gas chromatograph which was rented from Perkin-Elmer last fall. The first topic for study will be the relative amounts of Krebs cycle acids that accumulate in the medium during the growth of cells. The next study will use the technique of pyrrolysis gas chromatography to try to characterize cell cultures.

Study 3. Kinetics of growth of tumor cells and non-tumor cells. This study, in collaboration with Dr. J. A. Dvorak, will center on the growth kinetics of mammalian cells in culture. The research plan is fairly uncomplicated. Using as standardized conditions as possible, we will grow cells under an interference microscope and follow the acquisition of organic matter much in the way one would follow a batch culture of fungi or bacteria. The interference microscope will provide us with dry weight of biomass per unit time. This information will be amenable to thermodynamic calculations that will be done on a mini-computer which we have ordered. We intend to use synthetic media for study of incorporation of organic precursors into protoplasm, a study not as yet reported, with mammalian cells using these methods.

Study 4. In a collaborative study with O. W. McBride, we hope to be able to determine the rate at which chromosomes are incorporated into cells in culture. This study involves timelapse cinephotomicrography in which chromosome preparations are deposited on top of a culture of growing cells. We hope to be able to document the rate at which chromosomes are taken up by the cells, and possibly their fate as intracellular bodies. So far, three preliminary experiments have been run that have demonstrated considerable promise.

Publications:

1. Fox, C. H., Cottler-Fox, M. H., Sanford, K. K.: Rapid staining and preservation of cells grown in plastic tissue culture flasks. In press, Stain Technology.
2. Sanford, K. K., Handleman, S. L., Burris, J. F., Fox, C.H.: Effect of chemical carcinogens on neoplastic transformation and morphology of cells in culture. In press, J. Natl. Cancer Inst.

Serial No. NCI-5223
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Interaction between the first enzyme for histidine biosynthesis and His-tRNA

Previous Serial No.: None

Principal Investigator: Robert F. Goldberger

Other Investigators: Tikva Vogel, Roger Deeley and Marilyn Meyers

Cooperating Units: None

Man Years:
Total: 4
Professional: 2 1/2
Other: 1 1/2

Project Description:

Objectives: On the basis of our previous work on the kinetics of derepression of the histidine enzymes we postulated that the first enzyme for histidine biosynthesis plays a previously unrecognized role in repression of the histidine system in *Salmonella typhimurium*. Because it has been known for some time that His-tRNA is required for repression of the histidine system, it appeared to us that one test of our hypothesis is to determine whether the first enzyme for histidine biosynthesis interacts with His-tRNA. Using crude tRNA, we previously found that the enzyme had a high affinity for tRNA and that it bound His-tRNA in preference to tRNA which has been aminoacylated with any other amino acid. In the present study, we used various partially purified species of tRNA, to test the question of whether the enzyme binds the aminoacylated form of His-tRNA in preference to the deacylated form.

Methods Employed: Partially purified preparations of various species of tRNA were obtained from Dr. Donald Kelmers of the Oak Ridge National Laboratory. In addition, tRNA^{His} was purified to homogeneity from *Salmonella typhimurium* (both wild type and *T*-mutant) by the published procedure. The *G* enzyme and histidyl-tRNA synthetase were purified by the published procedures. Binding of the *G* enzyme to tRNA was studied by using the nitrocellulose filter binding technique of Yarus and Berg.

Major Findings: We found that for species of tRNA other than tRNA^{His}, the *G* enzyme does not distinguish between the aminoacylated and deacylated forms. In contrast, the *G* enzyme binds aminoacylated histidyl-tRNA in preference to deacylated $tRNA^{His}$. Thus, the enzyme not only binds the histidine species of tRNA in preference to all other species, but also binds the aminoacylated form of histidine tRNA preferentially. The possibility that histidyl-tRNA carries out its regulatory role as a complex with the G enzyme is consistent with these findings. Binding studies with His-tRNA from the T mutant demonstrated that the G enzyme does not differentiate between mutant and normal His-tRNA, in terms of its ability to bind either the acylated or deacylated forms of the tRNA when the enzyme is in either its active or feedback inhibited states.

Significance to Cancer Research: The finding that His-tRNA, a macromolecule known to be required for repression of the histidine system, binds with a very high affinity to the first enzyme of the histidine biosynthetic pathway, may be an important clue to understanding the mechanisms which regulate biosynthetic metabolic pathways.

Proposed Course of Research: Studies on the interaction between G enzyme and tRNA are being extended to examine the interaction of the G enzyme-tRNA complex with DNA of the histidine operon, to determine whether the regulatory defect in T mutants involves a defect in the ability of the altered His-tRNA G enzyme complex to bind to the DNA of the histidine operator.

Honors and Awards:
1. Invited Speaker, Canadian Biochemical Society International Symposium on Biochemical Control Mechanisms, Montreal, summer, 1973.
2. Invited Speaker, Gordon Conference on Nucleic Acids, New Hampshire, summer, 1973.
3. Session Chairman and Speaker, International Conference on Histidine Biosynthesis, Naples, summer, 1973.
4. DHEW Superior Service Award, fall 1973.
5. Chairman, Gordon Conference on Biological Regulatory Mechanisms, New Hampshire, (1973-74), Program Organizer.
6. American Society of Biological Chemists, winter, 1974.
7. Session Chairman, Biochemistry/Biophysics Meeting, Minneapolis, spring, 1974.

Publications:
1. Goldberger, R. F.: Autogenous regulation of gene expression. Science, 183: 810-816, 1974.
2. Goldberger, R. F. Deeley, R. G. and Mullinix, K. P. M.: Regulation of gene expression in prokaryotic organisms. In Ernst W. Caspari and Arnold W. Ravin (Eds.): Chapter in Genetic Organization, Vol. II. New York, Academic Press, 1974. In Press.

Serial No. NCI-5224
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The effect of transfer RNA on the activity of G enzyme, the first enzyme in the histidine pathway

Previous Serial No.: None

Principal Investigator: Robert F. Goldberger

Other Investigators: None

Cooperating Units: Henry Kronenberg, NIAMDD-LCB

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: Histidine inhibits the G enzyme, the first enzyme in the histidine biosynthetic pathway in *Salmonella typhimurium*. Charged $tRNA^{His}$ plays a role in repressing the histidine operon. G enzyme may play a role in gene regulation as well, and is known to bind specifically charged $tRNA^{His}$. We want to see if $tRNA^{His}$ has any specific effect on the activity of G enzyme itself.

Methods Employed: A sensitive new assay for G enzyme was developed because nucleotides interfere with the standard spectrophotometric method. The G enzyme catalyzes the reaction:

$$\text{PRPP} + \text{ATP} \qquad\qquad \text{PRATP} + \text{PPi}.$$

The assay uses the fact that C^{14} PRPP does not bind to charcoal at acid pH, but C^{14} PR-ATP does. Purified tRNA species were obtained by reverse phase chromatography and aminoacylated using standard techniques.

Major Findings: At very low concentration tRNA partially inhibits G enzyme. There was no inhibition of G enzyme from a mutant with altered G enzyme feedback sensitivity to histidine. Preliminary studies show no specificity for charged or uncharged $tRNA^{His}$. ATP and PRPP reverse both the binding and the inhibition by tRNA. Studies have been limited by the scarcity of charged $tRNA^{His}$. A new method will be used to prepare the tRNA.

Significance to Cancer Research: G enzyme--as the first enzyme in the histidine operon and pathway--may take part in histidine regulation on both the genetic and enzymatic level. Interaction with tRNA may shed light on the growing field of protein-nucleic acid interactions.

Proposed Course of Research: With newly available charged $tRNA^{His}$, the specificity and inhibition kinetics of the reaction will be determined, using normal and mutant G enzymes.

Honors and Awards: None

Publications: None

Serial No. NCI-5225
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Interaction between the first enzyme for histidine biosynthesis and 080dhis DNA

Previous Serial No.: None

Principal Investigator: Marilyn Meyers

Other Investigators: Tikva Vogel, Roger Deeley and Robert F. Goldberger

Cooperating Units: None

Man Years:
Total: 3/4
Professional: 3/4
Other: 0

Project Description:

Objectives: We previously proposed that the first enzyme for histidine biosynthesis, the G enzyme, plays a role in regulation of the histidine operon. If this enzyme acts at the genetic level, it would be expected to interact with some regulatory element in the DNA of the histidine operon. The recent isolation of a phage carrying the histidine operon in place of some of its own genes has made it possible to test this possibility.

Methods Employed: Radioactively labelled and unlabelled DNA were isolated from 080dhis. Interactions between these preparations of DNA and the G enzyme were studied by the filter binding assay used by Riggs and Bourgeois to study the binding of the lac repressor to the lactose operon. Competition experiments were done using cold 080 DNA, cold 080dhis DNA, and cold 080dhis DNA carrying a deletion mutation in the operator region of the his operon to determine if these preparations of DNA also bind to the G enzyme.

Major Findings: The enzyme binds 080dhis DNA. This binding is completely inhibited by cold 080dhis DNA. However, cold 080 DNA does not inhibit, and the cold 080dhis operator deletion DNA does not inhibit. These results indicate that the G enzyme binds to the regulatory region of the his operon DNA.

Significance to Cancer Research: The finding that an enzyme may serve as a regulatory protein controlling the rate of its own biosynthesis may be of general importance in understanding the mechanism of repression control.

Proposed Course of Research: We will study what effect on the binding of *G* enzyme to Ø80d*his* DNA is obtained by the addition of aminoacylated histidine tRNA and deacylated $tRNA^{His}$. We will also prepare radioactively labelled DNA from phages carrying the *his* operon with various mutations in the regulatory region (i.e., O^{c} and promoter mutations) to directly test the binding of the *G* enzyme to DNA carrying these alterations.

Honors and Awards: None

Publications: None

Serial No. NCI-5222
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mutations in the first structural gene of the histidine operon which alter regulation of the operon

Previous Serial No: None

Principal Investigator: Marilyn Meyers

Other Investigators: None

Cooperating Units: Mark Levinthal, Department of Biological Sciences, Purdue University

Man Years:
Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives: On the basis of previous work done on the kinetics of repression of the histidine operon in Salmonella typhimurium, we suggested that the first enzyme for histidine biosynthesis plays a previously unrecognized role in control of the histidine operon. A prediction of this hypothesis is that certain mutations in the first structural gene (G gene) of the operon would lead to alterations in the structure of the first enzyme which affect regulation of the histidine operon. Although a number of mutations affecting the catalytic function of this enzyme are known, none of these affect the process of repression. We have been attempting to find mutations in the first structural gene which do alter the repression process.

Methods Employed: Several selection procedures have been used. As reported previously, initial attempts to obtain mutations in the G gene involved selecting histidine auxotrophs from a strain containing two normally functioning genes for each of the histidine biosynthetic enzymes except for the G gene which is represented only once. Theoretically, all histidine auxotrophs derived from this strain should have mutations in the G gene. Although this method has led to the isolation of several strains bearing mutations in the G gene, this has been an extremely rare event, and none of these auxotrophs shows any change in the process of repression.

To be certain that every organism selected for study contains a new mutation in the G gene another procedure was tried. A large number of prototrophic revertants of several different complete G mutants were examined for alterations in regulation of the histidine operon. None was found to have a regulatory defect.

A third approach has been the selection of feedback resistant mutants, mutants in which the G enzyme is catalytically active but insensitive to inhibition by histidine. We had previously demonstrated that such mutants are altered in their ability to be repressed. To further study this phenomenon, we developed a procedure which allowed the direct selection of feedback resistant mutation in the G gene of episomes carrying the histidine operon. Twenty feedback resistant episomes were isolated. Several were found to produce a G enzyme highly resistant to inhibition by histidine. Using these episomes to make merodiploid organisms, we were able to demonstrate that the effect of the G enzyme on repression of the histidine operon was trans dominant (see Project Report NIAMDD-LCB-14). An exciting finding of these studies was that one of the feedback resistant episomes is not under repression control. This strain may be the first representative of the class of constitutive G gene mutations predicted by our hypothesis.

This episome has been integrated into the chromosome of several strains carrying deletions of the histidine operons. The resulting strains exhibit the same patterns, i.e., the levels of the histidine enzymes are approximately 3X derepressed and do not appear to be under histidine control. The episome carrying the altered G gene has been transduced into R, T. U, and W mutants of Salmonella and in all cases the enzyme levels are fixed at the 3X level. The same result was obtained when the episome was transduced into an E. coli strain which had the His operon deleted.

We are now in the process of characterizing the mutation to prove that it is a single mutation in the G gene which causes the loss of regulatory function.

Major Findings: An episome bearing the histidine operon containing a mutation to feedback resistance has been isolated. The strain carrying this episome is not under repression control. This finding supports the hypothesis that the G enzyme plays a direct role in repression of the histidine operon.

Significance to Cancer Research: If alterations in the first enzyme for histidine biosynthesis which affect repression of the histidine operon are found, they will constitute proof of our hypothesis, that a biosynthetic enzyme may have regulatory properties which allow it to control its own synthesis as well as the synthesis of the other biosynthetic enzymes.

Proposed Course of Research: Terminate after publishing results.

Honors and Awards: None

Publications: None

Serial No. NCI-5226
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: *In vitro* transcription of the histidine operon

Previous Serial No.: None

Principal Investigator: Roger Deeley

Other Investigators: Robert F. Goldberger, Marilyn Meyers, Francesco Blasi, Allesandra Avitabile and Carmello Bruni, University of Naples, Naples, Italy

Cooperating Units: None

Man Years:
Total: 1 1/2
Professional: 3/4
Other: 3/4

Project Description:

Objectives: An *in vitro* system was developed for transcription of the histidine operon of *E. coli* carried in the genome of a defective Ø80 transducing phage in order to study regulation of expression of the operon in a more simplified system than that provided by the intact bacterial cell *in vivo*.

Methods Employed: DNA was prepared from Ø80 and Ø80d*his* and the strands of these DNA preparations were separated by standard techniques. *In vitro* transcription was carried out by the method described by de Crombrugghe, with slight modifications. Tritiated UTP was included in the incubation mixtures so that the RNA synthesized *in vitro* would be radioactively labelled. RNA-DNA hybridization techniques were used to determine complementarity of the RNA made *in vitro* to the individual strands of Ø80 and Ø80d*his*. Sucrose density gradient centrifugation was utilized as a means for determining the size distribution of the RNA species synthesized *in vitro* from the phage DNA template.

Major Findings: Messenger RNA of the histidine operon was synthesized *in vitro*. This RNA was detected by hybridization to single strands of both Ø80 and Ø80d*his* DNA, and by competition of this hybridization with unlabelled histidine messenger RNA which had been synthesized *in vivo*. Under the conditions employed, histidine operon RNA amounted to about 15% of the total RNA which was synthesized *in vitro* from the Ø80d*his* DNA template. The RNA complementary to the histidine operon was synthesized on the "sense" strand (the R strand) in the form of a polycistronic message with a sedimentation coefficient (about 38S) very close to that observed for the histidine messenger RNA synthesized *in vivo*. Synthesis of the histidine operon RNA appears to be subject to control *in vitro*.

Significance to Cancer Research: The development of an *in vitro* system for studying regulation of a biosynthetic system will provide the means for determining the mechanism of such regulation. This mechanism has bearing on many problems of interest in the biomedical sciences.

Proposed Course of Research: The *in vitro* system will be used to determine the effects of histidine transfer ribonucleic acid, the first enzyme for histidine biosynthesis, and other molecules known, or suspected, to play a role in regulating expression of the histidine operon.

Honors and Awards: None

Publications:

Blasi, F., Bruni, C. B., Avitabile, A., Deeley, R. G., Goldberger, R. F., and Meyers, M.: Inhibition of transcription of the histidine operon *in vitro* by the first enzyme of the histidine pathway. *Proc. Nat. Acad. Sci. USA* 70: 2692-2696, 1973.

Serial No. NCI-5227
1. Laboratory of Biochemistry
2. Cellular Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In vitro synthesis of the enzymes of the histidine operon

Previous Serial No.: None

Principal Investigator: Kathleen Mullinix

Other Investigators: Roger Deeley, Robert Goldberger and Marilyn Meyers

Cooperating Units: None

Man Years:
Total: 2
Professional: 1 1/2
Other: 1/2

Project Description:

Objectives: In order to study regulation of expression of the histidine operon, we want to develop an in vitro system for the transcription and translation of the operon. An in vitro system requiring the synthesis of enzymes of the operon in an active form is the most rigorous test of specific RNA and protein synthesis, at the same time offering more opportunities for studying regulation than are available in studies on intact cells.

Methods Employed: Cell-free extracts of strains of Escherichia coli and Salmonella typhimurium having deletions of the histidine operon were made using modifications of the procedures of Zubay et al. DNA, to be used as a template for the in vitro synthesis, was prepared from the phages Ø80 and Ø80dhis and from various mutant phage strains. Active enzymes of the histidine operon were assayed according to procedures developed in this laboratory for the determination of activities of the enzymes of the first two genes of the operon, G and D. In addition, extracts have been prepared from E. coli having deletions of the lactose operon in order to test for production of β-galactosidase synthesized from the DNA of the phage λdlac.

Major Findings: It has been possible to prepare extracts of E. coli which synthesize β-galactosidase, in agreement with the findings of many other workers. It has also been possible to prepare extracts of E. coli which make low levels of the enzymes of the histidine operon. E. coli is not the system of choice, however, since the mutant strains necessary to study regulation are not available as they are in Salmonella. Thus far, it has not been possible to prepare extracts of S. typhimurium which can synthesize either β-galactosidase or the enzymes of the histidine operon. Preliminary studies have shown that extracts of Salmonella contain an inhibitor which probably acts by degrading the DNA which is used as a template in the reaction. Studies are now in progress to fractionate the extracts to remove any such inhibitors and genetic methods are being used to construct strains of Salmonella which can be used for cell-free synthesis (having deletions of the histidine operon) and which will be free of molecules which are inhibitory to transcription and translation.

Significance to Cancer Research: The development of in vitro systems for synthesis of enzymes of the histidine operon will provide an opportunity for elucidating the mechanism of regulation of a biosynthetic operon. Regulation of biosynthesis is clearly one of the major problems of interest in the biomedical sciences.

Proposed Course of Research: After the initial goal of development of an in vitro transcription-translation system in Salmonella is reached, we will be able to determine in vitro the effects of proteins and small molecules known, or suspected, to play a role in regulation of expression of the histidine operon.

Honors and Awards: None

Publications: None

Serial No. NCI-5236
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
March 4, 1974 through June 30, 1974

Project Title: Restriction Endonuclease and Modification Methylase of Methylase of coliphage P1

Principal Investigator: Maxine F. Singer, Ph.D.

Other Investigators: None

Cooperating Units: Judah L. Rosner, Ph.D.
Laboratory of Molecular Biology
National Institute of Arthritis, Metabolism,
and Digestive Diseases

Man Years

Total:	0.3
Professional:	0.1
Other:	0.1

Project Description:

Objectives:

To study the presence and absence of active P-1 specific restriction endonuclease and modification methylase in cells infected with relevant mutants of P-1 coliphage. To purify and study the properties of these two enzymes.

Methods Employed:

A variety of mutants made available by Dr. Rosner's genetic studies are allowed to form gene products in appropriate cells. Extracts are made and fractionated by standard techniques including chromatography. Enzyme assays utilize radioisotope tracers. Preparative ultracentrifugal techniques and gel electrophoresis are used. P-1 modified and unmodified phage DNAs are prepared for use as substrates.

Major Findings:

Except for the consistent observation that the two enzymes are extremely difficult to measure reliably in crude extracts or partially purified preparations no significant findings have been made. The

Major Findings: (Continued)

difficulties have at least two explanations: 1) the instability of both enzyme activities and 2) interference in the assays by other specific and nonspecific host (*E. coli*) endonucleases and methylases.

Significance to Biomedical Research and the Program of the Institute:

The restriction and modification enzymes have proved of enormous analytical utility in studies of DNA structure and metabolism. Further, restriction and modification (endonuclease and paired methylase) is a major mechanism whereby bacteria protect themselves from infection by bacteriophage and preserve their own DNA. It is widely believed that similar, but as yet unknown processes, are present in mammalian cells.

Proposed Course of Project:

To "overcome" the current difficulties and proceed with the stated objectives.

Honors and Awards:

1974 Award for Advancement of Basic and Applied Science

Yale Science and Engineering Association

Publications:

None

Serial No. NCI-5232
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
February 1, 1974 through June 30, 1974

Project Title: Studies on the Replication of Simian Virus 40 (SV40) DNA in Monkey Kidney Cells

Principal Investigator: Donald J. LeBlanc, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

1. To determine the cellular location of SV40 DNA synthesis.

2. To develop an in vitro replication system which closely approximates the in vivo situation.

3. To utilize an in vitro replication system to study the enzymology of SV40 DNA synthesis.

Methods Employed:

Standard tissue culture procedures for the growth of monkey kidney cells and SV40. Adaptation of published procedures for the isolation of cell nuclei and nuclear membrane-associated DNA. Procedures used for studying viral DNA synthesis included radioisotope tracer techniques, preparative and analytical ultracentrifugation, DNA-DNA hybridization and selective precipitation of high molecular weight DNA.

Major Findings:

Radioisotope tracer studies confirmed earlier observations by electron microscopy indicating that SV40 DNA synthesis occurs in the nuclei of infected cells. The so-called "M-band" technique was used to initiate studies on the site of SV40 DNA replication in the host nucleus. The

Major Findings: (Continued)

results suggest that the majority of the radioactive precursor incorporated into SV40 DNA during short pulses is associated with the host nuclear membrane. The species of SV40DNA which has been defined as replicative intermediate appears to be associated with the membrane. Preliminary results suggest that mature SV40 DNA is released from the membrane upon completion of replication.

Significance to NCI Research:

Simian Virus 40 is an oncogenic DNA virus known to produce tumors in hamsters. Experimental evidence shedding light on the mode of replication of this virus is of obvious significance to NCI research.

Proposed Course of Project:

Attempts to elucidate further the function of the DNA-membrane complex and to confirm the association of replicating SV40 DNA with host nuclear membrane by other methods will be made. In addition, this complex will be used to initiate *in vitro* studies on the synthesis of SV40 DNA with the aim of elucidating the enzymology of the replicative process.

Publications:

None

Serial No. NCI-5237
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
January 1, 1974 through June 30, 1974

Project Title: Replication of Simian Virus 40 DNA in Monkey Kidney Cells

Principal Investigator: Tikva Vogel, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total:	0.65
Professional:	0.55
Other:	0.1

Project Description:

Objectives:

1. To study the DNases associated with the conversion of the closed supercoiled form of SV40 DNA into the nicked relaxed form. This reaction is proposed to be required for initiation of viral DNA replication and recombination.

2. To study the interaction between mammalian histones and SV40 DNA *in vitro* and *in vivo*.

Methods Employed:

Standard tissue culture techniques for growth of monkey kidney cells and SV40 (Simian Virus 40) adaptation of published assays for measuring DNase activity. Subcellular fractionation, column chromatography, radioisotopes tracer techniques, gel electrophoresis, preperative and analytical ultracentrifugation, millipore binding technique.

Major Findings:

We developed a sensitive assay for measuring a single strand scission of the closed supercoiled molecule of SV40 DNA (nickase activity). This assay depends on two facts: 1) nicked molecules are irreversibly denatured under conditions in which the closed circular supercoiled molecules are not denatured and 2) denatured DNA is specifically hydrolyzed by the S_1

Major Findings: (Continued)

nuclease of A. oryzae. We then used a DEAE-cellulose fractionation of extracts prepared from nuclei, cytoplasm and whole cells from both infected and noninfected monkey cells. Nickase activity was measured in the different fractions by the S_1 technique and confirmed by ultracentrifugal analysis of products.

We confirmed the existence of nickase activities in nuclear extract or cytoplasmic extract of both infected and noninfected monkey cells. The various activities observed in each case, after DEAE-cellulose chromatography were essentially identical, in giving 3 major regions of nickase activity.

The first nickase activity eluted from the columns with the wash fluid, and was the major activity. All the characteristics of the activity in this fraction were identical for infected and noninfected cells, and were similar to the nickase activity previously detected in isolated SV40 virions.

The second area of nickase activity elucted from the column with low salt, and was similar to the activity in the wash. Again no differences were observed between infected and noninfected cells. The third area of nickase activity eluted from the column with high salt; the activity was minor in amount. The activity was associated with some proteins which were able to interact with SV40 DNA in vitro, and markedly increase the sedimentation rate of the DNA. Thus, the proteins bind to the DNA. These proteins were found exclusively in nuclear extracts. These proteins mimic in some ways the effects of nuclear histones, but our results are not yet conclusive and work is still inprogress.

Significance to NCI Research:

SV40 is an oncogenic virus known to induce tumor in hamsters. Thus, studying the steps induce in viral replication are of obvious importance.

Proposed Course of Project:

1. To compare further the nickase activity of SV40 virions and the nickase activity of the column wash fraction obtained from infected and noninfected extracts, thus to study whether SV40 virions incorporate pre-existing cellular nickase.

2. To study the nature of the nickase activity eluted from the column with high salt and found to be associated with nuclear proteins. We will try to separate the nickase and the other proteins physically and study each one independently as well as the relation between them.

3. To continue our in vitro studies on the interaction between viral DNA and fractionated histones.

Proposed Course of Project: (Continued)

4. To relate those *in vitro* studies to *in vivo* studies by determining which histones are bound to SV40 DNA in isolated SV40 virions.

Publications:

None

Serial No. NCI-5234
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
January 1, 1974 through June 30, 1974

Project Title: Replication and Properties of Defective-Substituted Simian Virus 40 in Permissive Monkey Kidney Cells.

Principal Investigator: G. R. Koteswara Rao, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total:	0.55
Professional:	0.45
Other:	0.1

Project Description:

Objectives:

Serial, undiluted passage of Simian Virus 40 (SV40) yields defective virions containing DNA which is 1) shorter than normal 2) partially substituted with host sequences. We wish to study the preferential replication of such defective-substituted DNA as well as the nature of the host DNA sequences incorporated into SV40.

Methods Employed:

Standard tissue culture procedures are used for the growth of monkey kidney cells and SV40. Procedures used for studying viral DNA synthesis included radioisotope tracer techniques, preparative and analytical ultracentrifugation, DNA-DNA hybridization, selective precipitation of high molecular weight DNA, polyacrylamide and agarose gel electrophoresis, degradation with specific restriction endonucleases. Radioisotope tracer methods are essential.

Major Findings:

In order to study the mechanism of enrichment and replication of defective-substituted SV40, serial undiluted passages were continued in monkey kidney cells. With continuing passages the percent of host sequences in defective viral DNA increased and at the same time the number

Major Findings: (Continued)

of plaque-forming units in the passage yields decreased (4 orders of magnitude). Studies with Hemophilus influenzae restriction endonuclease confirmed earlier findings that the fragments produced by restriction enzyme cleavage are completely different from those obtained with the nondefective viral DNA. Thus, the defective DNA is drastically altered over most of the molecule.

Pulse-label experiments with $[^3H]$thymidine were used to measure the rate of DNA synthesis. Upon infection with the yields of later and later passages, the rate of DNA synthesis diminishes to background level.

At the 8th passage, the defective virus changed markedly. Suddenly the yield of plaque-forming units increased by about 3 orders of magnitude, the passage gives, upon infection of cells, a rapid cytopathological effect, and rapid viral DNA synthesis. Thus, less defective virus has been produced. Subsequent passaging again leads to more and more defective virus.

Highly defective passage yields markedly inhibit the rate of viral DNA synthesis when mixed with non-defective virus prior to infection. This inhibition is dependent on the concentration of infecting defective material and can be overcome by increasing the concentration of non-defective virus.

Significance to NCI Research:

Simian Virus 40 is an oncogenic DNA virus known to produce tumors in hamsters. Thus, any experimental evidence shedding light on the mode of replication of this virus is of obvious significance to NCI research. Further studies on substituted defective SV40 have 3 broad implications:

First they raise the possibility of transduction experiments in animal systems and second, the formation of the substituted molecules provides an interesting approach to the metabolism of DNA upon viral infections. Third, defective animal virions occur upon passage of many viruses, and it has been proposed that they play a part in the self-limitation of viral infections.

Proposed Course of Project:

We will try to investigate the mechanism whereby defective viral DNA is synthesized preferentially over non-defective DNA. Further, we are interested in understanding how substituted molecules arise. This will entail investigation of the nature of those host sequences which are incorporated into substituted SV40 DNA. We shall also carry out studies on the mechanism by which defective SV40 inhibits the replication of standard, non-defective SV40.

Serial No. NCI-5235
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
January 1, 1974 through June 30, 1974

Project Title: The Physiological Role of Polynucleotide Phosphorylase

Principal Investigator: G. R. Koteswara Rao, Ph.D.

Other Investigators: Maxine F. Singer, Ph.D.

Cooperating Units: None

Man Years

Total:	0.25
Professional:	0.15
Other:	0.1

Project Description:

Objectives:

To obtain evidence concerning the physiological role of polynucleotide phosphorylase. To date, no role has been unambiguously assigned to this ubiquitous enzyme although many speculations have been presented.

To investigate the possibility that poly A sequences are added to RNA molecules in *E. coli* as they are added to mRNA in eukaryote cells.

Methods Employed:

E. coli cells permeable to nucleotide substrates were prepared by toluene treatment. Techniques evolved previously for measuring polynucleotide phosphorylase reactions were used, as was affinity chromatography. Mutants either deficient in or containing modified polynucleotide phosphorylase were used. Density gradient centrifugation provided a means of determining polymer size. Specific degradation of polyribonucleotides was carried out with pancreatic and T_1 ribonucleases.

Major Findings:

E. coli A19 (RNase I^-) cells were made permeable to nucleotide substrates by toluene treatment. Using these cells with ADP as substrate, poly A was formed and purified by means of poly U-sepharose column chromatography. The size of the poly A was determined by neutral sucrose

Major Findings: (Continued)

density gradient centrifugation and HCHO-sucrose gradient centrifugation. Poly A made in these cells was heterogeneous in size ranging from 30S - 16S with a peak at around 25S. Polynucleotide phosphorylase deficient mutants were also used in these studies. No polymerization reaction was detected in these mutant cells but 15% of normal phosphorolysis activity was observed. Using another mutant which produces modified polynucleotide phosphorylase (Q13) no polymerization activity was observed with Mg^{2+} as cofactor and the poly A made with Mn^{2+} as cofactor was smaller in size (12S) than that made in A19 cells. Experiments were designed to detect any RNA species that might contain added poly A sequences. None of the poly A synthesized in the toluenized cells was covalently linked to previously labeled RNA.

Significance to NCI Research:

The methods employed and the results obtained in studies of nucleic acid metabolism in unicellular organisms contribute to the study of virus infected and normal animal cells.

Proposed Course of Project:

Terminated.

Publications:

None.

Serial No. NCI-5233
1. Laboratory of Biochemistry
2. Nucleic Acid Enzymology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In vitro Simian Virus 40 DNA synthesis

Previous Serial No.: None

Principal Investigator: Pradman K. Qasba

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: To understand the detailed enzymology and control mechanisms of DNA synthesis in eukaryotic cells. With isolated nuclei from mammalian cells attempts have been made to study various aspects of eukaryotic DNA synthesis. But there are inherent difficulties and serious limitations in these studies, such as the problems of characterizing the high molecular weight DNA and poorly defined nature of the synthetic product. For these reasons I chose Simian Virus 40 (SV40) - a simple mammalian DNA virus - known to replicate in nucleus as a tool for the study of eukaryotic DNA synthesis in vitro. This DNA can be easily separated and characterized from the bulk of the DNA and much is known about its replication in vivo.

Methods Employed: Isolation of nuclei from the cells. DNA synthesis in vitro with isolated nuclei in the presence of radioactively labeled TTP and UTP. Isolation of viral DNA by fractional precipitation. Characterization of synthesized product by sedimentation and density analysis and hybridization and reassociation kinetics.

Major Findings: Conditions were developed, where isolated nuclei from SV40 infected African green monkey kidney cells, could synthesize in vitro SV40 DNA. Under the conditions of in vitro synthesis, nuclei isolated from infected cells at different times after infection maintain the same temporal sequence of host and viral DNA synthesis as seen in intact cells. The viral DNA synthesized by the nuclei of cells previously infected for 32-35 hours was characterized by centrifugation through neutral and alkaline sucrose gradients, and by isopycnic banding in a propidium iodide-cesium chloride gradient. DNA synthesis in this system is maintained for only 5-7 minutes. Neutral sucrose gradient analysis showed that most of the radioactivity is associated with replicative intermediate of SV40 DNA and the rest sediments at 5-7 S. Alkaline gradient analysis showed that 50-60% of the radioactivity sediments as 3-7 S fragments, and the rest between 7 and 16 S pulse-chase experiments showed that in this system 3-7 S fragments do not mature into long chains. These results have been submitted for publication in Proc. Nat. Acad. Sci. USA.

RNA linked to SV40 DNA synthesized _in vitro_. Radioactivity derived from $[^3H]$ UTP is found associated with the replicative intermediate isolated on Benzoylated napthoylated DEAE cellulose and on Cs_2So_4 gradient. The labeled material bands at the density of DNA on Cs_2So_4 gradient even after heat denaturation. The radio active material banding at the DNA density is degraded by RNAase and by alkali. The results indicate that RNA is covalently linked to SV40 DNA. At present I am writing these results for publication.

Characterization of 5-7 S DNA and replicative intermediate. In an alkaline gradient about 45% of the radioactivity associated with the replicative intermediate sediments as 3-7 S DNA and the rest between 7-16 S. 5-7 S DNA fragments isolated from neutral gradients sediment entirely as 3-7 S DNA in an alkaline gradient. 5-7 S DNA hybridizes with unlabeled SV40 DNA with an efficiency of 95%, when under similar conditions of hybridization $[^3H]$ SV40 DNA hybridized to 63% efficiency. Analysis on hydroxylapatite and BND-Columns shows that 30-40% of the 5-7 S DNA eluted as single-stranded and the rest as double-stranded DNA. S_1 nuclease digests both single-strand and double-strand fractions from these columns to 85-90% acid soluble counts. The results suggest an unusual structure of 5-7 S DNA. These results are also being written for publication.

Proposed Course of Research: At present I am studying the factors involved in the maturation of the DNA and the role of Nicotinamide adenine denucleotide during DNA replication.

Significance to Cancer Research: This system will enable us to find the enzymes involved during DNA replication and in particular during the replication of SV40 DNA which is a tumorogenic virus.

Honors and Awards: None

Publications:

Pradman K. Qasba: Synthesis of Simian Virus 40 DNA in isolated nuclei. _Proc. Nat. Acad. Sci. USA_ (in press).

Serial No. NCI-5231
1. Biochemistry
2. Nucleic Acid Enzymology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
January 20, 1974 through June 30, 1974

Project Title: Role of Subunit Interactions in Enzyme Chemistry

Previous Serial Number: None

Principal Investigator: Dr. Claude B. Klee

Other Investigators: None

Cooperating Units: Drs. Werner Klee and K. Subramonia Iyer, Laboratory of General and Comparative Biochemistry, National Institute of Mental Health
Drs. Louis A. Cohen and Kenneth L. Kirk, Laboratory of Chemistry, and Dr. Peter McPhie, Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Metabolism, and Digestive Diseases

Man Years:

Total:	0.7
Professional:	0.5
Others:	0.2

Project Description:

Objectives:

To identify the chemical structure of the active site residue of histidine ammonia-lyase and the participation of the enzyme subunits in the formation of the catalytic center. To re-initiate studies on the role of subunit structure and interactions in the initiation of polynucleotide synthesis catalyzed by polynucleotide phosphorylase.

Methods Employed:

The project involves the study of properties of proteins, purified by the usual techniques of protein isolation, including many types of chromatography. The enzymes are being studied by examining their optical, hydrodynamic, and kinetic properties as well as by suitable chemical measurements and modifications.

Major Findings:

I. *Histidine Ammonia-Lyase.* Histidine ammonia-lyase catalyzes an α,β elimination of NH_3 from histidine to produce urocanic acid. The active center of the enzyme contains an electrophilic center, as yet not characterized, which interacts with the α amino group of the substrate. Our present studies are directed toward the elucidation of the structure of the active center and of the mechanism of the elimination reaction.

Active Site Structural Studies. Active site labeling of histidase with $Na[^3H]BH_4$ results in the incorporation of 3-4 moles of ^3H/mole of enzyme tetramer, and 50% of the bound ^3H is recovered as $[^3H]$alanine after acid hydrolysis. Exposure of the labeled enzyme to denaturing conditions prior to acid hydrolysis resulted in a loss of $[^3H]$alanine (< 0.4 moles/mole of enzyme), indicating that the dehydroalanine postulated at the active center is not simply linked to the protein by a peptide linkage.

We previously described a stereospecific inactivation of the enzyme by L-cysteine which is accompanied by the binding of 3.2-3.5 moles of cysteine per mole of enzyme tetramer and by the subsequent appearance of a new absorption peak at 340 nm. The protection observed in the presence of L-histidine, urocanate, but not D-histidine, indicates that the modification is active site specific. Preliminary evidence indicates that cysteine acts as a substrate analog interacting with the enzyme through its amino group. Binding of cysteine is followed by a chemical change responsible for the new absorption peak. The structure responsible for this absorption does not include the added cysteine since the cysteine may be dissociated from the enzyme under denaturing conditions or extremes of pH without loss in absorption at 340 nm. A 10-15 residues peptide containing the new chromophore but not the labeled cysteine has been purified and is presently being analyzed. A Schiff base between the amino group of dehydroalanine and an unknown carbonyl function has been postulated by Givot, Smith, and Abeles. It is possible that such a carbonyl group may be involved in the reactions of the enzyme with cysteine, but further studies are required before any such inferences may be confidently made.

Mechanism of Action. Our investigation of the β elimination of NH_3 from L-histidine and 4-fluoro-L-histidine by histidine ammonia-lyase has been extended to include $4\text{-}NO_2$-L-histidine. Under comparable conditions the V_m for L-histidine, 4-fluoro-L-histidine, and $4\text{-}NO_2$-L-histidine were 20, 0.5, and 1.7 μmole/min/mg, respectively, and the K_m were not significantly different. The steady state kinetics previously reported indicated that the rate determining step precedes the dissociation of ammonia from the enzyme. An isotope effect of 1.5 to 1.8 observed with $[\beta\text{-}^2H_2]$-DL-histidine and $[\beta\text{-}^2H_2]$-4-fluoro-DL-histidine suggests that the C-H bond breakage is at least partially rate determining for these two substrates. No isotope effect is observed in the deamination of $[\beta\text{-}^2H_2]\text{-}4\text{-}NO_2$-DL-histidine in which the β hydrogen is labilized by the substituent in the 4 position of the imidazole ring. The rate limiting of the deamination of $4\text{-}NO_2$-L-histidine is therefore different from that of histidine or 4-fluoro-histidine. This conclusion is confirmed by the absence of inhibition by EDTA observed with 4-NO_2-histidine in contrast to the metal activation of the deamination of L-histidine which has been attributed to the labilization of the β-hydrogen.

II. *Lactose Synthetase*. Complete guanidination of α-lactalbumin, the "regulatory subunit" of lactose synthetase, can be achieved without significant change of conformation or activity in specifying lactose synthesis.

Significance to Biomedical Research and the Program of the Institute:

Histidine ammonia-lyase and phenylalanine ammonia-lyase are the only enzymes so far known to have unsaturated amino acid residues as part of their active sites. Doubtless, other such enzymes will ultimately be described and an understanding of the mechanism of action of the one protein will be of use in understanding the mode of action of the whole class. The mechanisms by which such unsaturated amino acids arise, both in proteins and in some peptide antibiotics, are open questions but may well have implications for the structure of some cross-linked proteins. Dehydroamino acids formed as a result of the elimination of various groups on the β carbon of amino acid residues may be important in structural as well as functional roles.

Proposed Course:

The study of lactose synthetase has been terminated. Future work on histidine ammonia-lyase will be directed toward the characterization of the chemical structure of the electrophilic center and of its function in the mechanism of the catalysis of the α,β elimination. Structure function relations of polynucleotide phosphorylase, more particularly the mechanism of the initiation of polynucleotide synthesis and the processive degradation of nucleic acids, will be re-initiated in the coming year.

Honors and Awards: None

Publications: None

Serial No. NCI-375
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Homogeneity and structure of proteins

Previous Serial No.: Same

Principal Investigator: Elbert A. Peterson

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1 2/3
Professional: 2/3
Other: 1

Project Description:

Objectives: To develop methods for the separation and analysis of proteins that will overcome present limitations and to apply these methods to the purification of specific proteins for the study of their function and structure.

Methods Employed: A variety of polyanions and polycations differing in molecular size, charge density, and chemical composition are prepared by appropriate reactions. Most of these are ionic derivatives of low molecular weight dextrans but other types are being explored. They are evaluated as displacing agents, using test protein mixtures, on cellulosic ion exchangers. Effluent fractions are examined by electrophoresis in slabs of polyacrylamide gel and by appropriate assay.

Major Findings: Following an extensive study of the behavior of displacement systems in the absence of protein samples (which complicate the measurement of phenomena resulting from the interaction of displacers with the adsorbent), a procedure has been devised for the use of the displacers in protein separations which appears to provide adequate control of both pH and level of displaced counter-ions. These were previously disturbing factors.

Additional CM-dextrans, of different carboxyl content and molecular weight, have been prepared and methods for their isolation have been further improved. Also, new procedures for characterizing these displacers with respect to carboxyl content and relative affinity for DEAE-cellulose are being developed.

Significance to Cancer Research: Displacement chromatography of proteins, nucleic acids, etc. promises to be of considerable value at any scale of operation and therefore has significance to all research that involves the isolation of such substances. Its immediate significance to the program of this Section, however, lies in its potential application to the fractionation of the small quantities of protein available in each fraction obtained when bone marrow cells are separated into populations of different cell types. The displacers, unlike salt, do not interfere with electrophoresis, and protein fractions obtained by displacement can be directly subjected to electrophoresis or rechromatography without dialysis or concentration. Thus the examination of complex mixtures by a combination of chromatography and electrophoresis on a semi-micro scale is greatly expedited. The simplicity of operation also facilitates automation, so that the two-dimensional analysis of large numbers of fractions becomes feasible.

Moreover, since migration is induced by displacers under conditions of salt and pH in which the migrating molecules are otherwise strongly adsorbed, the possibility arises of chromatographing substances that are "irreversibly" absorbed in conventional procedures. Moreover, theoretical considerations strongly suggest that many of the enzymes that become labile when purified can be protected from inactivation or loss by substances such as the CM-dextrans if the metal-sequestering properties of the system are taken into account.

Proposed Course of Research: The method, as it develops, will be applied to the subfractionation of t-RNA and to the examination of proteins and nucleic acids in guinea pig bone marrow cells. Its possible application in the separation of cells and subcellular organelles will be explored, using appropriate adsorbent matrices. Also, a stabilizing effect of the CM-dextrans will be looked for using enzymes that have previously been difficult to purify because of their high lability.

Honors and Awards: None

Publications: None

Serial No. NCI-5202
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Isolation, fractionation, and characterization of native nucleoproteins

Previous Serial No.: Same

Principal Investigator: O. Wesley McBride

Other Investigators: John B. Burch, Raghbir S. Athwal, Miles C. Otey, Michael G. Mage, Elbert A. Peterson

Cooperating Units: None

Man Years:
Total: 4
Professional: 3 1/2
Other: 1/2

Project Description:

Objectives: The determination of the role of nucleic acid-bound proteins in the regulation of the transcription and translation of genetic information, and the biological transformation of cells with isolated and fractionated mammalian metaphase chromosomes as a means of genetic mapping.

Methods Employed: Tissue culture procedures involving propagation of mammalian cells in monolayer and suspension cultures, plating in selective media, cloning, labelling with radioisotopes, selection of cell mutants, and metaphase arrest for chromosome isolation. Metaphase chromosome isolation and purification by isopycnic and velocity sedimentation (including unit gravity procedures), and evaluation of chromosomal DNA molecular weight by zonal density gradient sedimentation. Gene transfer to mammalian cells via uptake of purified metaphase chromosomes and isolation of biologically transformed colonies in selective media. Isolation, quantitation, and characterization of gene products by chromatography, electrophoresis, isoelectric focusing, and immunochemical procedures.

Major Findings: The system for assay of gene transfer from isolated metaphase chromosomes to mammalian cells has been modified. Since the recipient L_{A9} cells lack both Adenyl and Hypoxanthine Phosphoribosyl Transferases (APRT and HGPRT), it is now possible to simultaneously assess the transfer of two different genes (one sex-linked and the other autosomal) by employing HAAT (hypoxanthine, adenine, amethopterin, and thymidine) as the selective growth medium. Under these conditions, several clones have been isolated containing an active APRT as well as several more with active HGPRT, but no clones have been found to contain both transferases. The transferases from APRT$^+$ clones are currently being characterized to exclude the possibility that these clones simply arose through a back-mutation in some recipient cells.

Several colonies with an active adenyl or hypoxanthine PRTase have also been obtained in experiments utilizing human chromosomes and recipient L_{A9} cells, and enzyme extracts from these clones are being characterized biochemically. The HGPRT gene product from at least one clone has been identified as chromosomal donor species while the APRT from two other clones was found to be mouse (revertant) species. Enzyme extracts from HGPRT transformants are also being examined for the presence of other x-linked, chromosomal donor, marker genes (glucose-6 phosphate dehydrogenase, phosphoglycerokinase, and α-galactosidase) but no definitive results are available yet.

Biochemical studies indicate that HGPRT of both Chinese hamster and mouse species is strongly inhibited by mercuribenzoate (PCMB) as well as several other thiol reagents. The substrate, MgPRPP, provides complete protection against PCMB inhibition and partial protection from inactivation by the other reagents. The inhibition of HGPRT by PCMB can be partially reversed by subsequent incubation with thiols but the extent varies according to the species of origin of the enzyme. The reversibility is much greater for mouse than hamster species, i.e. 50% vs. 0-10% under mildest conditions up to 90-100% vs 40-50% for more drastic conditions. These results seem to reflect actual differences in the two species of HGPRT rather than differences in some other component of the crude extracts. Using the criterion of reversibility of PCMB inhibition, extracts from transformants resemble the hamster donor species whereas those from revertants resemble the mouse recipient species. This may be another useful criterion for identifying the species of HGPRT present in extracts.

Purification of HGPRT would greatly facilitate many of the current studies and partial success has resulted from conventional DEAE- and CM-cellulose chromatography. An affinity adsorbent has been prepared by covalently attaching the ribose moiety of 5'-IMP to Sepharose through amino-hexyl sidearms. The HGPRT is adsorbed very strongly on columns of this material and the column-bound enzyme remains active enzymatically. Most proteins, including APRT, are eluted under these same conditions. Adsorption of HGPRT does not occur in the presence of free IMP or the substrate MgPRPP. Work is in progress to establish the optimal concentrations of IMP or MgPRPP, as well as monovalent and divalent cations, to specifically elute HGPRT.

Proposed Course of Research: Studies will be completed which demonstrate transfer of the functioning APRT gene from metaphase chromosomes to recipient cells, thereby providing evidence suggesting the generality of gene transfer by this procedure. More HGPRT-positive clones, resulting from studies with human chromosomes and L_{A9} cells, will be analyzed for the presence of other x-linked marker enzymes to determine the amount of information transferred, i.e., entire chromosome or free or integrated chromosome fragments. This will be supplemented by karyological analysis via Giemsa banding and constitutive heterochromatin staining.

The gene products will be further characterized. Purification of the PRTases by affinity chromatography and other methods will permit identification of the species of origin by peptide mapping of proteolytic digests. Efforts continue to obtain species-specific antibody to the PRTases.

Molecular hybridization of DNA from transformant clonal lines with that from the parental hamster and mouse lines should strengthen evidence for gene transfer and possibly permit some quantitation of this process. Depending on these results, in situ DNA hybridization on slides will be considered as a means of localizing the transferred DNA to a particular chromosome.

Isolated chromosomes will be fractionated according to size, density, charge, and surface properties by unit gravity sedimentation, isopycnic centrifugation, ion-exchange chromatography on suitable adsorbents, and partitioning in solutions of aqueous polymers. These fractionated chromosomes are especially important for genetic mapping and investigating sites of integration of oncogenic viruses.

Significance to Cancer Research: Metaphase chromosome isolation and fractionation provides the most promising approach for the fractionation of mammalian genes and can provide material for the investigation of control elements associated with specific sets of genes. The biological expression of these chromosomes introduced into recipient cells provides a method for mapping of mammalian genes, investigating differences between genetic elements in normal and neoplastic cells, and looking for sites of integration of oncogenic viruses into the mammalian genome.

Honors and Awards: None

Publications:

McBride, O. W.: Expression of foreign chromosomal genes following uptake of isolated metaphase chromosomes by mammalian cells. In Redei, G.P. (ed): Stadler Genetics Symposium, Columbia, Mo., University of Missouri, 1974, Vol. 6 (in press).

Serial No. NCI-333
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The biochemical basis for defective maturation in myelogenous leukemia

Previous Serial No.: Same

Principal Investigator: Warren H. Evans

Other Investigators: John Noseworthy, Michael Mage, Elbert A. Peterson, Shirley Wilson, Margaret Wolf, Bruce A. Chabner and C. Norman Coleman

Cooperating Units: Laboratory of Chemical Pharmacology

Man Years:
Total: 5
Professional: 5
Other: 0

Project Description:

Objectives: 1) To identify the main molecular constituents and metabolic processes which distinguish mature granulocytes from their immature precursors. 2) To determine whether the major structural and biochemical changes associated with granulocyte maturation are regulated by factors present in body fluids and/or by cell-mediated factors. 3) To use the information gained from (1) and (2) to; (a) identify the primary molecular abnormalities associated with the arrested maturation of granulocytes in acute and chronic myelogenous leukemia and possibly develop methods for the early diagnosis of leukemia, (b) develop specific biological and/or biochemical methods for inducing leukemic cells to develop some or all of the functional characteristics of mature granulocytes as a means of partial or complete restoration of host defense mechanisms in leukemia patients.

Methods Employed: Leukocytes are isolated from bone marrow, blood, and inflammatory exudates by a variety of methods. Subcellular fractions are prepared by differential and zonal centrifugation and further fractionated by chromatography and electrophoresis. Immunological reagents, including fluorescent antibodies, are prepared from the purified fractions and used to follow the development of related material during maturation of the cells. The maturation of enriched cell populations is studied in soft agar and stationary tissue culture. The incorporation of radioactive precursors into proteins and nucleic acids of cells is studied, using liquid scintillation counting methods.

Major Findings:

1) Role of Arginase in Granulocyte Maturation. Our studies indicate that arginase may play a role in the conversion of myelocytes to mature neutrophil granulocytes. We have observed that enriched populations of mature cells have at least 5-6 times the arginase activity of enriched populations of immature granulocytes and the enzyme appears to be localized primarily in the extragranular portion of the mature cell. We have also shown that the conversion of immature cells to mature granulocytes in short-term cultures (24 hours) in vitro is accompanied by 2-3 fold increase in arginase activity.

2) Changes in Cell Surface Antigens during Granulocyte Maturation. We have obtained evidence that the cell surface antigens of granulocytes undergo marked changes in vivo and in vitro. Using fluorescent antibody and cell separation techniques we have demonstrated that mature granulocytes from guinea pig bone marrow possess surface antigens which are absent on immature cells. We have also shown that these antigens appear on the surface of mature granulocytes formed from immature marrow cells incubated in short-term culture in vitro. The nature and function of these surface antigens are unknown at present.

3) Protein Profiles of Granule and Cytosol Proteins in Mature and Immature Granulocytes. Work continues on the development of methods for separating the primary and secondary lysosome-like granules of mature granulocytes. Differential centrifugation of sucrose homogenates of such cells has permitted significant relative enrichment of primary granules and secondary granules as determined by specific enzymatic analysis of the pellets. However a significant degree of contamination of the granule pellets with cell membrane material does exist, as documented by both enzymatic (5'-nucleotidase activity) and morphologic (electronmicroscopy) criteria. At present, methods of eliminating this membrane contamination are being pursued. The major protein constituents of the soluble cytoplasm of mature and immature granulocytes have also been compared using gel electrophoresis. Unlike the postnuclear particulate fraction which showed a marked increase in glycoprotein during cell maturation, the soluble fraction appears to undergo relatively little change with respect to the major protein constituents.

4) Cytidine Deaminase in Human Bone Marrow Granulocytes. Since we hope to apply our findings obtained with guinea pig bone marrow granulocytes to studies of human bone marrow, we have developed a procedure for isolating enriched populations of mature and immature granulocytes from normal human bone marrow. To our knowledge this is the first such method for human marrow which provides separated cells in sufficient quantity and purity for biochemical studies. The technique, based on Ficoll density centrifugation, yields one fraction containing 76% immature granulocytes and only 9% mature cells. This fraction is being used in studies of the role of cytidine deaminase in the development of resistance in leukemic patients to the drug cytosine arabinoside. We have found markedly lower (3-4 fold) levels of deaminase activity in lysates of normal immature cells compared to normal mature cells. A similarly decreased enzyme activity was found in immature granulocytes from patients with chronic

myelogenous leukemia compared to mature cells from the same patients. These observations support the conclusion that the greater deaminase activity which has been reported for normal blood granulocytes compared to that of leukemic cells is related to the process of cell maturation rather than reflecting a specific enzymatic defect in leukemic cells. The need for cell separation according to cell maturity in studies using mixed cell population is indicated by this work.

Significance to Cancer Research: Understanding of normal and leukemic granulocyte maturation depends upon clarification of the molecular processes whereby the cell controls the appearance and disappearance of specific components. Such information has never been obtained at the biochemical level in a systematic fashion similar to that achieved in morphological studies of normal and leukemic granulocytes. Any biochemical differences in the maturation processes observed between normal and leukemic leukocytes will be exploited for purposes of developing techniques, presently unavailable, for the early diagnosis of leukemia and for improved treatment, especially with regard to the possibility of reversing the arrested maturation and decreased phagocytic activity of leukocytes from myelogenous leukemia.

Proposed Course of Research: The factors that regulate arginase activity in marrow granulocytes remain to be identified. We plan to investigate the possibility that this enzyme is regulated by adrenal cortical steroids as has been shown for liver arginase. The role of cyclic nucleotides in the regulation of arginase activity will also be studied. In addition attempts will be made to determine whether leukemic myeloblasts, which are known to lack arginase, can be induced to mature *in vitro* in an arginine-deficient medium.

Studies are underway to determine precisely the maturation stage at which arginase first appears in bone marrow granulocytes. Since no histochemical method for arginase exists at present, we plan to use fluorescent antibody techniques to detect the enzyme in smear preparations of cells. We also hope to be able to determine by this method whether arginase is associated with cell nuclei.

We also plan to determine the subcellular localization of the glycoproteins that we showed previously to be associated with the late stages of granulocyte development. Further purification of the granule and membrane fractions of mature granulocytes will be required for this study.

Honors and Awards: None

Publications:

1. Huang, F. L. and Evans, W. H.: Differences in protein and glycoprotein patterns between mature and immature neutrophil granulocytes. *Biochim. Biophys. Acta* 317: 394-402, 1973.

2. Chabner, B. A., Johns, D. G., Coleman, C. N., Drake, J. C. and Evans, W.

H.: Cytidine deaminase from leukemic granulocytes: purification, properties and comparison with enzymes from normal granulocytes. J. Clin. Invest. 53: 922-931, 1974.

Serial No. NCI-5203
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of immunochemical and physical methods for the purification and characterization of immunocytes and their subcellular components

Previous Serial No.: Same

Principal Investigator: Michael G. Mage

Other Investigators: Louise McHugh

Cooperating Units: None

Man Years:
Total: 1 1/2
Professional: 1 1/2
Other: 0

Project Description:

Objectives: The goal of this project is the development of methods for the specific isolation of immune cells, particularly for antigen-reactive cells involved in cellular immune reactions, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules.

Methods Employed: Antigen-reactive lymphocytes (ARC) are obtained from the spleens of normal mice and from mice immunized with allogeneic tumor cells and normal cells. Spleen cell suspensions containing ARC are incubated on foam columns coated with allogeneic and syngeneic target cells. The nonadherent cells are tested for depletion of cytotoxic activity by measurement of ^{51}Cr release from target cells. Graft-versus-host (GVH) activity is measured by the Simonsen spleen weight assay in neonatal mice.

Major Findings: Our finding that GVH activity is retained after removal of cytotoxic effector cells (by absorption to allogeneic target cell monolayers), initially obtained on incubation of BALB/c spleen cells on C57Bl/6 x BALB/c F_1 monolayers, has been also obtained on incubation of spleen cells from the other parental strain (C57Bl/6) on the same F_1 monolayers, confirming the original observation. Following up on this finding, we are attempting to find conditions under which the GVH-ARC as well as the cytotoxic effector cells can be bound to target cells. Current experimentation involves the use of target cells bound to foam columns as cellular immunoabsorbents.

Significance to Cancer Research: These studies on the specific separation of antigen-reactive cells relate to the mechanisms of cell-mediated tumor rejection. Knowledge of the antigen-binding characteristics of immune cells may help in development of specific purifications of immune lymphocytes with antitumor reactivities, and may aid in understanding the variation in tumor susceptibility of human beings and make possible control of immune mechanisms of tumor surveillance and rejection.

The GVH reaction is an iatrogenic complication of tumor therapy regimens that include bone-marrow transplantation. A specific separation of GVH-antigen reactive cells might aid in avoiding this complication.

Our reason for working with antigen-coated foam columns (which we have used to specifically bind antibody-producing cells) is that these columns have high binding capacity (on the order of 10^8 cells/cc) and thus have the potential for preparative scale separations of immune lymphocytes.

Proposed Course of Research: As an aid to the development of general methods for the specific isolation of antigen-reactive lymphocytes, we plan to further study the antigen-binding characteristics of immune T-cells, particularly with respect to target-cell antigen specificity, the conditions needed for antigen-binding by ARC, and the effect on antigen-binding of enzyme-treatment of target and/or immune-cell surfaces.

Honors and Awards: None

Publications:

Mage, M.G., and McHugh, L.L.: Retention of graft-vs-host activity in non-adherent spleen cells after depletion of cytotoxic activity by incubation on allogeneic target cells. J. Immun. 111: 651-655, 1973.

Serial No. NCI-5230
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical properties of neoplastic cell membranes

Previous Serial No.: None

Principal Investigator: Dante Marciani

Other Investigators: John P. Bader and Elbert A. Peterson

Cooperating Units: Chemistry Branch, DCCP, NCI

Man Years:
Total: 2/3
Professional: 2/3
Other: 0

Project Description:

Objectives: The study of the modifications of membrane components, mainly proteins, that occur in cells transformed by a temperature-sensitive mutant of Rous Sarcoma virus: the study of the role of these modifications in properties of the cell that are expressed at, or mediated by, the cell surface.

Methods Employed: Isolation of cell membranes by differential and density gradient centrifugation. Analysis of the membrane composition by electrophoretic and chromatographic techniques. Study of the surface topography by use of enzymic labeling with ^{125}I or tritium, combined with electrophoresis. Determination of receptors for radioactively labeled lectins.

Major Findings: Studies during the past year were concerned primarily with plasma membranes from chicken fibroblasts, normal and transformed by a temperature-sensitive mutant (Ta) from Rous sarcoma virus isolated by Dr. J. Bader.

(1) Electrophoretic analysis in polyacrylamide gels containing sodium dodecyl sulfate and urea has shown that cells transformed by the wild type of Rous sarcoma virus (Bryan) present differences from normal chicken fibroblasts. On the other hand, cells transformed with the Ta from Rous sarcoma virus present electrophoretic patterns almost identical to those of normal fibroblasts. Electrophoretic analysis in a phenol-urea-acetic acid system reveals differences between normal and transformed cells. Differences are also observed between membranes isolated, respectively, from cells growing at the permissive and nonpermissive temperatures for transformation.

(2) For studies utilizing the binding of lectins to detect changes in the outer surface of the cell membrane, (^{3}H) concanavalin-A has been prepared by acetylation with (^{3}H)-acetic anhydride. Also a new method has been developed for the preparation of highly purified hyaluronidase, free of protease activity. This enzyme is required for the treatment of cells, prior to the binding of lectins, in order to remove interfering hyaluronic acid.

Proposed Course of Research: We are planning to study the changes in surface topography as a function of temperature shift. These studies will involve measurements of agglutination by lectins, and determination of the number and nature of lectin receptors.

Study of possible modifications of membrane components, such as phosphorylations and changes in glycoproteins, using radioactive precursors and electrophoretic methods. These changes will be studied as a function of temperature and correlated with other altered properties of transformed cells, such as glucose and water transport.

Dr. Bader has infected hamster cells with his Rous sarcoma temperature-sensitive mutant. We are planning to see if the same changes observed in chicken cells happen in mammalian cells.

Significance to Cancer Research: The fact that the sociology of cells is mediated through their cell surfaces makes the study of cell membranes important for understanding the lack of contact inhibition, the changes in permeability, and the capacity for invading normal tissue that are characteristic of neoplasic cells.

Honors and Awards: None

Publications: None

PHS-NIH
Summary Report
July 1, 1973 through June 30, 1974
Laboratory of Biology
NCI

The Mammary Tumor System

The main thrust of the Laboratory of Biology is on mammary gland tumors, the transmission of the causative factors and mammary tumorigenesis. The mammary tumor of the mouse with its mammary tumor virus are studied as a model system for the eventual control of breast cancer in women. Supportive work has also been carried on with the avian myeloblastosis virus and in the areas of biochemical genetics and cell biology.

Dr. G. Smith, collaborating with Dr. R. Gantt and Dr. D. Longfellow, is interested in the intracellular events in mammary tumorigenesis, particularly the nature of the intracytoplasmic A particle that has been thought to be the nucleoprotein core of the mammary tumor virus (MTV). Although MTV is an RNA virus, Dr. Smith has observed a high molecular weight DNA unique to these intracytoplasmic A particles. He has also found a low molecular weight RNA associated with these A particles in contrast with the high molecular weight RNA isolated from MTV. In characterization and isolation studies he has further compared these A particles with the MTV B particles. Biologic studies indicate that the purified A particles are not infective.

Mr. Vlahakis and Dr. Heston have through a study of foster-nursed mouse strains noted differences in response to different lines of MTV when given to the same strain of mice and differences in response of different strains of mice to the same line of MTV. There is a question as to whether all strains of mice have a line of MTV. In testing extracts of mammary glands from old BALB/c females, Vlahakis has failed to confirm the presence of any MTV.

For studying the genetics of the transfer of MTV in crosses between strain C3H-A^{vy}fB with male transmission of an MTV and C57BL presumably without MTV, Dr. Hoffman in collaboration with Dr. Grieshaber has been developing methods for the immunofluorescence absorption test for the MTV group specific antigen in the mammary gland. Whole glands appeared negative for the antigen, but they have now developed a technique for enzymatic dissociation of mammary gland and mammary tumor epithelial cells and have been able to detect antigen in the dissociated cells.

One of the greatest recent advances in mammary tumor research is Bentvelzen's hypothesis of genetic transmission of the MTV. Critical is the proposal that the viral information is transmitted as a single locus on the chromosome, yet reports on this point are conflicting. This can be settled only through a breeding test of backcross segregants and Dr. Heston has now set up this test from crosses between strain GR with the presumably genetically transmitted MTV and C57BL without MTV. Dr. Wade Parks is collaborating in this work in assaying the milk samples from the hybrids for MTV antigen.

Dr. Stromberg has divided his time during the past year between Dr. Spiegelman's Cancer Research Laboratory and Dr. King's Pathology Laboratory at Columbia where he has continued his work on Avian Myeloblastosis Virus (AMV). He has been able by exposure of the intact virion to nonionic surfactant to separate the viral "envelope" from the viral "core" and then by exposure of the viral cores to high concentration of sodium chloride to release the inner viral RNP, or "nucleoid." He has then characterized these three components by electron microscopy, and by biochemical, enzymatic, and biologic methods. These procedures of fractionation and characterization can now be applied to human material to see if there is a possible association between Type-C viruses and cancer in man. Because the enzymatic and polypeptide assays such as he has used for AMV can be made to be highly specific and extremely sensitive, he may be able to develop tests for early detection of cancer in high-risk human populations. He plans to use this approach also for detecting breast cancer in women and its possible causative virus.

Biochemical Genetics

Drs. Hoffman and Grieshaber continue to build up their information on the biochemical genetics of the mouse working particularly with catalase. Dr. Grieshaber has developed a method for isolation of erythrocyte catalase. They have isolated erythrocyte catalase from BALB/c mice and human beings. The two appear to be indistinguishable by immunochemical methods, but the preparation from humans has a specific activity of 6.8×10^7 M^{-1} sec^{-1} whereas that from the mice has a specific activity of 2.7×10^7 M^{-1} sec^{-1}. A twofold difference in RBC catalase between BALB/c and C57BL/6 mice has been shown to be a difference in concentration of catalase in the cell rather than a structural difference in catalase. They have shown two isozyme patterns for kidney catalase, the C57BL/6 (Pattern B) and C3H (Pattern A). Genetic analyses indicate control by a single locus with B dominant to A and linked to H-2 on chromosome 17.

In collaboration with Dr. Hilgers of Amsterdam, Hoffman has constructed biochemical genetic profiles of many of the inbred strains of mice used in cancer research. This is valuable basic information on these strains.

Cellular Biology

Dr. Longfellow in collaboration with Drs. Smith and Gantt has studied DNA methylation in paired normal and neoplastic mouse cell lines *in vitro* and found that the principal DNA methylation is 5-methylcytosine, but they have also found with more sensitive techniques a significant level of another tentatively identified as 1-methylguanine. Major amounts of newly synthesized DNA and DNA polymerase are found with fractions of the nucleus which contain the highest levels of these methylase activities. Now they can compare the DNA methylation activities of the normal and neoplastic cells to determine if there is an increase in DNA methylation associated with the neoplastic process as reported for RNA methylation.

Ovarian Carcinomatous Ascites

Dr. Feldman came to the laboratory last July 1 with a definite problem in mind on ovarian carcinomatous ascites. He had two objectives: 1) to determine the role of lymphatic obstruction in the etiology of the ascites, and 2) to determine the influence of anti-coagulants and fibrinolytics on preventing the ascites. Utilizing an ascitic forming ovarian tumor of the mouse, he demonstrated that the ovarian tumor cells did not enter the lymphatic channels and in this way block the drainage of the peritoneal cavity thus causing the ascites, but that the blockage occurred as a result of layering out of tumor cells over the drainage surface of the peritoneum. He found, as one might then expect, that neither systemic heparin, systemic coumarin, nor intraperitoneal heparin inhibited the formation of the ascites. This would suggest their ineffectiveness in preventing ovarian carcinomatous ascites in women.

Test for Carcinogenesis of Cedar Shavings

Sabine has reported that in high hepatoma and high mammary tumor strain C3H-A^{vy} and C3H-A^{vy}fB mice obtained from this laboratory and maintained in his laboratory in Australia, these tumors failed to occur. In a paper in *JNCI* he has attributed the difference to the fact that we use cedar shavings in our cages. This reflects seriously on much of the work done in this country where cedar shavings have been used and raises the questions as to whether cedar wood and cedar wood products are carcinogenic to man. We are now testing the two obvious differences, the difference in diet and the cedar shavings vs. pine shavings alone. Results thus far indicate that the diet was not the factor causing the difference. Furthermore, our mice on pine shavings alone are getting tumors just as those on cedar and pine. We will have the final data this fall. It would appear that his failure to get tumors was due to the poor husbandry of his animals.

Serial No. NCI-3208
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Gene Regulation of Protein Turnover and Its Relationship to Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Harold A. Hoffman

Other Investigators: Charles K. Grieshaber
Warren Evans (Laboratory of Biochemistry, NCI)
Jo Hilgers (Netherlands Cancer Institute)
Hisami Ikeda and Edward Boyse (Sloan Kettering Inst.)

Cooperating Units: Office of Program Resources and Logistics, DCCP, NCI (Supplied rabbit anti-MTV serum)

Man Years:

Total:	5.4
Professional:	2
Others:	3.4

Project Description: Objective 3. Approach 3

Objectives: To study by methods appropriate to biochemical and quantitative genetics the gene regulation of protein synthesis and degradation in the mouse and other mammals and to determine the genetic and non-genetic relationships between protein metabolism and tumorigenesis. To determine the primary structure of the normal protein and the aberrant or mutant protein in order to define the gene or genes controlling their production and to determine the time of gene action during the process of neoplastic transformation.

Methods: In these investigations mice from 18 inbred strains and sub-strains, which represent at least six different independent lines of known genetic origin, are used as sources of proteins and tumors, and for the production of allotypic antiserum. New Zealand white rabbits of known immunoglobulin type are used for the production of heterologous antiserum, and non-human primates of the order anthropoid are used as a source of protein.

Immunochemical methods, agar-gel double diffusion, immuno-electrophoresis, and immunofluorescent absorption, are used in the detection and characterization of different proteins. Physical and chemical techniques of gel electrophoresis and chromatography are used to isolate and classify different proteins. The fine structure and minute differences between closely related proteins are determined by peptide map analysis (fingerprints) and amino acid analysis.

Major Findings: Inheritance of tissue and erythrocyte catalase in inbred mice. Using an isolation technique developed in the laboratory, catalase was purified from BALB/c erythrocytes and from human erythrocytes. The specific activity of human RBC catalase was 6.8×10^7 M^{-1} sec^{-1} and the specific activity for the murine catalase was 1.7×10^7 M^{-1} sec^{-1}. The purified murine catalase was used to produce a monospecific rabbit anti-catalase serum. Immunoelectrophoretic analysis of BALB/c and C57BL/6 RBC lysates demonstrated a single major catalase protein which is identical with respect to electrophoretic mobility in both strains. Thus, the difference in RBC enzyme activity between BALB/c and C57BL/6, 108 and 83 units per gram of hemoglobin, respectively, is probably due to differences in enzyme concentration in the cell rather than structural differences between BALB/c and C57BL/6 catalase.

Two congenic inbred lines for acatalasemia have been established for BALB/c and C57BL/6; the congenic lines are in their second generation of brother by sister inbreeding after 7 backcross generations to their respective inbred strains. The F_1 hybrids from the cross between V57BL/6 and its congenic strain B6.CN exhibited over-dominance for liver catalase activity; the liver value for C57BL/6 was 98 units/gm liver, for B6.CN 75 units/gm liver, and for the F_1 hybrid (C57BL/6 x B6.CN) 141 units/gm liver. There was no corresponding effect in kidney or erythrocyte catalase.

A second biochemical difference for murine catalase has been found; there are two isozyme patterns for kidney catalase. The type strains for the two patterns are C57BL/6 (Pattern B) and C3H (Pattern A). A genetic analysis, including F_1 hybrids and first backcross segregants, indicated control by a single autosomal locus with two alleles, where allele B is dominant to allele A. The kidney isozyme locus appears to be linked to the H-2 locus--H-2K end--on chromosome 17. There is no relationship between the level of kidney catalase and isozyme pattern; the two isozyme types are equally distributed among the high and low activity strains.

Biochemical markers in inbred strains of mice used in cancer research: A joint program with Dr. J. Hilgers to construct a genetic profile of the inbred strains of mice used in cancer research was recently initiated. To date, strains from the NCI, the Netherlands Cancer Institute, and Cancer Research Genetics Laboratory of the University of California, have been analyzed. Attached are two tables comparing 14 loci.

A similar survey for 23 loci among five sublines of AKR in which two sublines are low leukemic strains, showed that the high and low leukemic sublines differed at five loci thus indicating that outcrossing of the two low leukemic strains is the most likely reason for the AKR variation.

Inheritance of the expression of MTV group specific (gs) antigens: Virgin C3H, C3H-A^{vy}, C3H-A^{vy}fB and GR females, 6-12 weeks of age, did not express MTV-gs in their mammary glands. The immunochemical test employed was immunofluorescent absorption (IFA) and whole mammary glands were used as a source of antigen. Since whole glands were used, the ratio of mammary epithelium to fat pad may be very small and therefore may be the reason for the negative results with IFA. To determine if this is the case, we developed a method for enzymatic dissociation of mammary gland and mammary tumor cells. Using dissociated mammary gland epithelial cells from a multiparous C3H-A^{vy}fB female, we were able to detect gs-antigen by immunofluorescence (IF). Dissociated mammary tumor cells in the above inbred strains were all positive for gs-antigen by IF.

Significance for Cancer Research: (NCP Objective 3, Approach 3)

This study provides an animal model for the determination of genetic and non-genetic factors for human breast cancer.

Proposed Course of Project: Isolation of murine kidney catalase isozyme type A and type B will be carried out using the procedure developed for erythrocyte catalase. The isolated enzymes will be characterized for possible structural differences and for specific activities. Linkage tests have been initiated to determine the exact location of the kidney isozyme locus with respect to the H-2 locus. The rabbit anti-RBC catalase reagent will be used to determine by quantitative immunoprecipitation if differences in catalase activity between the inbred strains is due to concentration differences of the enzyme in the cell or due to differences of affinity of the enzyme for its substrate.

Dissociated mammary gland epithelial cells will be used in a time course study for the appearance of MTV-gs and NIV-gs antigen in C3H-A^{vy}fB females and (C3H-A^{vy}fB x C57BL)F_1 hybrids and backcross segregants. Both unprimed and estradiol-deoxycorticosterone primed females will be used.

Publications:

Acton, R., Blankenhorn, E., Douglas, T., Owen, R., Hilgers, J., Hoffman, H., Boyse, E.: Variations among sublines of inbred AKR mice. Nature 245: 8-10, 1973.

Grieshaber, C. and Hoffman, H. A rapid and convenient method for isolation of murine erythrocyte catalase by DEAE-cellulose chromatography and gel filtration. Anal. Biochem., in press.

Chromosome	1	1	4	5	7	8	9	11
Locus	Dip-1	Id-1	Gpd-1	Pgm-1	Gpi-1	Es-1	Mod-1	Es-3
A/Crgl	b	a	b	a	a	b	a	c
BALB/cCrgl	a	a	b	a	a	b	a	a
C3H/Crgl	b	a	b	b	b	b	a	c
C3Hf/Crgl	b	a	b	b	b	b	a	c
C57BL/Crgl	a	a	a	a	b	a	b	a
GR/Crgl	b	a	b	b	b	b	a	b
I/Crgl	b	a	b	b	a	b	b	c
GR/AHf	b	a	b	b	b	b	a	b
I/An	b	*	b	*	a	b	b	c
C57BL/6Hf	a	a	a	a	b	a	b	a
BALB/cHf	a	a	b	a	a	b	a	a
C3Hf/Hf	b	a	b	b	b	b	a	c

*Not tested

Strain	Chromosome Locus	Dip-1	Id-1	Gpd-1	Mup-1	Pgm-1	Ldr-1	Gpi-1	Hbb	Es-1	Mod-1	Es-3	Hc	Kidney Catalase	Kidney Esterase
A/BrA		b	a	b	a	a	a	a	d	b	a	c	-	B6	B6
Af/A		b	a	b	a	a	a	a	d	b	a	c	-	B6	B6
A2G/Ga		b	a	c	a	b	a	a	d	b	a	c	-	B6	B6
BALB/cCrg/A		a	a	b	a	a	a	a	d	b	a	a	+	B6	B6
BIMA/A		a	a	a	b	a	a	b	s	a	b	a	+	B6	B6
BIR/A		a	a	a	b	a	a	b	s	a	b	a	+	B6	B6
C3H/HeA		b	a	b	a	b	a	b	d	b	a	c	+	C3	B6
C3Hf/A		b	a	b	a	b	a	b	d	b	a	c	+	C3	B6
DBA/LiA		b	b	a	a	b	a	a	d	b	a	c	+	B6	B6
DBAf/A		b	b	a	a	b	a	a	d	b	a	c	+	B6	B6
DBA/HeA		b	b	b	a	b	a	a	d	b	a	c	-	B6	B6
LIS/A		b	a	b	b	b	a	b	d	b	b	c	-	B6	ST
LTS/A		b	a	b	a	b	b	b	d	b	a	c	-	B6	ST
LTSf/A		b	a	b	a	b	b	b	d	b	a	c	-	B6	ST
OIR/A		b	a	a	b	b	a	b	d	b	a	c	-	B6	B6
P/A		a	a	a	b	a	a	b	s	a	NT*	a	NT	B6	B6
TSI/A		b	a	b	b	b	b	a	d	b	a	c	+	B6	B6
WLL/BrA		c	a	a	a	b	a	a	d	b	a	c	+	B6	B6
WLLcB/A		c	a	a	a	b	a	a	d	b	a	c	+	B6	B6

*NT = Not tested

Serial No. NCI-3251
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Effect of Diet and Bedding (Cedar Shavings) on Occurrence of Mammary Tumors and Hepatomas in Mice

Principal Investigator: W. E. Heston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description: Objective 1. Approach 4

Objective: To analyze the cause for the startling observation made by Dr. Sabine in Australia that C3H, C3H-A^{vy} amd C3H-A^{vy}fB mice in his colony derived from our colony of these high hepatoma and high mammary tumor strains failed to develop hepatomas and mammary tumors in his laboratory in Australia.

Methods: Two obvious factors by which the two colonies differed had to be tested to see if they caused the difference in occurrence of hepatomas and mammary tumors in the laboratory in Australia as compared with their occurrence in our NCI laboratory. 1) Sabine fed his mice Charlick's diet, a mouse diet manufactured in Australia whereas our colonies were fed Purina Laboratory Chow or our NIH diet. 2) Sabine did not use cedar shavings in his bedding whereas we kept our mice on a mixture of cedar and pine shavings. We, therefore, set aside a group of C3H-A^{vy} females fed Charlick's diet to be compared in respect to occurrence of mammary tumors with littermate control females fed Purina Laboratory Chow. We also set aside a group of C3H-A^{vy} males and one of C3H-A^{vy}fB males to be compared in respect to incidence of hepatomas at one year of age with littermate controls fed Purina Laboratory Chow.

To test whether the cedar shavings were the influencing factor we split our breeding colony of C3H-A^{vy} mice keeping one line on cedar and pine shavings and the other line on pine alone. We also set aside a group of C3H-A^{vy} virgin females on cedar to compare with littermate virgin females on pine in respect to mammary tumor incidence. Also a group of C3H-A^{vy} males have been placed on cedar and pine to compare their hepatoma incidence with that of littermate males on pine alone.

Results: The Charlick food was not the factor causing the reduction in tumors in the laboratory in Australia, in fact the Charlick's diet increased the tumor incidence and/or shortened the tumor age from that observed in those fed Purina Laboratory Chow. This difference was directly related to a weight difference, the mice on Charlick's growing somewhat faster than those fed Purina Chow possibly because the Charlick's diet was softer and more easily eaten.

Final results for the difference in bedding are not yet available but preliminary results would indicate that the difference in tumors is not due to the cedar shavings either. It appears that all females on either the cedar and pine or the pine alone will develop mammary tumors and to date the average tumor ages of the two groups are identical. We will start autopsying the males at 12 months to look for hepatomas in April.

Significance to the Program of the Institute: Sabine has published results which he believes indicate the difference in tumors in the two laboratories is due to our using the cedar shavings thus implicating cedar as a general carcinogen. This, if true, would have a great impact on interpretation of many results published from NCI where cedar shavings have had general use. It would make people throughout the world who have used cedar wood, cedar oil, or other cedar wood products very much concerned as to whether they have been exposed to a carcinogen. The results we are getting should allay these fears.

The absence of tumors in the mice in Australia is probably due to poor husbandry. Cedar is used to keep off ectoparasites. Sabine's mice became badly infested with ectoparasites and weighed considerably less than ours. This would cause them to have fewer tumors.

Proposed Course: The project will be continued until all mice have come to autopsy, the data have been fully analyzed, and the results published. This should be completed during the coming year.

Serial No. NCI-3254
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization of a Representative Animal Type-C RNA Tumor Virus, Avian Myeloblastosis Virus (AMV), in Order to Develop Methods for Detection of Similar Viral Information in Human Tissues

Previous Serial Number: Same

Principal Investigator: Kurt J. Stromberg, M.D.

Man Years:

Total:	4
Professional:	1
Other:	3

Project Description:

I. Isolation of the AMV core component. II. Identification and localization of enzymatic capacities in the AMV virion. III. RNA and polypeptide composition of the AMV core component. IV. Determination of the biologic capacity of AMV core component. V. Fractionation of the AMV core component and characterization of its ribonucleoprotein (RNP) constituents. VI. Application of these methods to detection of type-C particle information in human cancer tissue.

National Cancer Plan: Objective 5, Approach 4

Methods and Results:

The experimental design has been to fractionate AMV into its component parts by two successive methods: firstly, by exposure of intact AMV to a nonionic surfactant which separates the viral "envelope" from the viral "core"; and secondly, by exposure of the resulting viral cores to high concentrations of sodium chloride which releases the inner viral RNP, or "nucleoid." The three viral components are separated by ultracentrifugation and then characterized by electron microscopic, biochemical, enzymatic methods, and biologic methods.

The core component of AMV contains 86% protein, 8% RNA, and 6% phospholipid as compared to 62% protein, 3% RNA and 35% phospholipid in the virion of AMV. The AMV core does not contain measurable glycoprotein or a major 22,000 M.W. structural polypeptide which are found in intact AMV preparations. The presence of most of the transfer RNA (tRNA) in the core component, in addition to the 60-70S RNA genome, has been determined, and suggests that tRNA species may be involved in the viral life cycle. SDS-polyacrylamide gel electrophoresis indicates the AMV core does not contain glycoprotein, or a major 22,000 M.W. polypeptide which are found in virion preparations of AMV. Polypeptides of 91,000 and 64,000 M.W. appear to correspond to the two subunits detected in preparations of purified AMV DNA polymerase; this, then, identifies which of the approximately 20 proteins present in intact AMV are responsible for reverse transcriptase activity. Our studies indicate that a major 14,000 M.W. polypeptide is the core protein in association with the 60-70S RNA genome of the RNP constituent of the virion. Recent work has focused on a method for selective release and partial purification of the DNA polymerase activity of the core component of AMV by exposure to sodium chloride. A greater than 10-fold purification in this reverse transcriptase activity is rapidly achieved in this manner which, in addition, releases those several polypeptides which appear to comprise the inner ribonucleoprotein of the AMV core. The presence of a small, but consistent, amount of DNA has been identified in AMV cores. However, preparations of AMV cores are not leukemogenic when injected into the peritoneal cavities of neonatal chicks of a susceptible genetic strain.

Significance of this Project to the Institute:

Early detection of preclinical cancer in man represents a major objective of current cancer research. Rapid, simple, and quantitative methods have been developed for separation of the envelope, core, and RNP components of a representative C-type RNA tumor virus. The possible association of type-C RNA tumor viruses in human cancer dictate that the structural, biochemical, and enzymatic characteristics of animal type-C viruses be determined as a prerequisite to detection of similar viral-like characteristics in human fluids and precancerous tissues. As an example, the unique and important advantage of salt treatment of AMV cores is that the DNA polymerase-containing core is separated from possible non-viral contaminants and membrane components before release of the DNA polymerase by sodium chloride. This procedure, therefore, may assist in the detection of viral particle-associated DNA polymerase activity in crude biological materials such as tumor extracts, samples of milk, etc. These procedures of fractionation and characterization can now be applied on human material to glean information on the possible association of type-C viruses to cancer in man. Because the enzymatic and polypeptide assays can be made to be highly specific and extremely sensitive, it may be possible to develop tests for early detection of cancer in high-risk human populations.

Publications:

Stromberg, K. and Litwack, M.D.: Structural studies of avian myeloblastosis virus: Presence of transfer RNA in core component. Biochim. Biophys. Acta 319: 140-152, 1973.

Stromberg, K., Hurley, N. E., Davis, N. L., Rueckert, R. R., and Fleissner, E.: Structural studies of avian myeloblastosis virus: Comparison of polypeptides in virion and core component by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Virol. 13: 513-528, 1974.

Stromberg, K. and Wilson, S. H.: Structural studies of avian myeloblastosis virus: Release of DNA polymerase activity and selective polypeptides from the core component by exposure to sodium chloride. Biochim. Biophys. Acta, in press.

Serial No. NCI-3256
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ultrastructure, Biology, Biochemistry, and Immunology of Mammary Tumor Virus Host-Cell Interaction

Previous Serial Number: Same

Principal Investigator: Gilbert H. Smith

Other Investigators: David G. Longfellow
R. Raymond Gantt

Man Years:

Total:	3-1/2
Professional:	1-1/2
Other:	2

Project Description: The objective of the project is to elucidate the aspects of cell-virus interaction in mammary tumor virus (MTV) infection and tumorigenesis. The approach or methods involve the isolation and purification of intracytoplasmic A type particles (putative MTV nucleoprotein cores) from primary and transplanted tumors and the purification of B particles (mature MTV virions) from primary mouse mammary tumors. Biochemical, immunologic and biologic analysis and comparison of these two types of particles are pursued in an attempt to establish an ontologic relationship between them and to assess their relative role in mammary neoplasia in mice.

Major Findings: High molecular weight DNA unique to intracytoplasmic A particles [the putative nucleoprotein cores of an RNA tumor virus (MTV)]. A publication describing these findings has been submitted to Cancer Research for publication.

Low molecular weight RNA associated with purified A particles (in addition to the DNA described above) in contrast to high molecular weight (60-70S) RNA isolated from identically purified M MTV. Studies are in progress to characterize this RNA and to establish its relationship to A particle DNA and MTV RNA.

Characterization of the A particle DNA including its strandedness, buoyant density, base composition, labeling characteristics and its appearance under the electron microscope. A manuscript describing these results is in preparation.

Isolation and characterization of A particles and their proteins by SDS polyacrylamide gel electrophoresis. Comparison of these results with those obtained with MTV under identical conditions. These results were published in the Journal of Virology.

Biologic studies relating to the infectivity and tumorigenic capacity of purified intracytoplasmic A particles have continued and are near completion. The results indicate that these particles are biologically inactive in this respect. A manuscript describing these experiments is planned.

Incorporation studies with ^{3}H-thymidine in intracytoplasmic A particle-containing transplantable testicular tumors were assessed by light and electron microscope autoradiography. Statistical analysis of grain distribution was carried out. Significant incorporation into A particle inclusions was observed. Follow-up studies to demonstrate the specificity of the label are planned.

Significance for Cancer Research: (NCP Objective 3. Approach 3)

The mouse mammary tumor virus problem has been and is being used as a relevant model for understanding human mammary neoplasia. Yet our knowledge of the intracellular events in MTV infection is essentially nil. The project is designed to provide a base to further our understanding of the intracellular events with the aim of elucidating the replicative cycle of MTV. Subsequently it is hoped, armed with this information, to specify the tumorigenic influence of the virus.

Publications:

Smith, G. H. and Wivel, N. A.: Intracytoplasmic A particles: mouse mammary tumor virus nucleoprotein cores. I. Comparison of A particle structural proteins with those of mouse mammary tumor virus. J. Virol. 11: 574-584, 1973.

Gantt, R. R., Smith, G. H., and Julian, B. T.: Base specific methylase activity in RNA tumor viruses: Avian leukosis virion-associated RNA methylase(s). Virology 53: 584-586, 1973.

Serial No. NCI-3260
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Part I. Genetic Transmission of the Mammary Tumor Virus in Mice

Previous Serial Number: Same

Principal Investigator: W. E. Heston

Other Investigators: Wade Parks

Man Years:

Total:	1-1/2
Professional:	1
Other:	1-1/2

Project Description: Objective 1. Approach 1

Objective: The aim of this project is to ascertain whether or not the mammary tumor virus information is transmitted as a single gene in the mouse.

Methods: The critical test for answering this question, the breeding test of backcross segregants will be carried out. The high tumor strain GR has been obtained from Amsterdam and strain GR males have been outcrossed to strain C57BL females. The F_1 males in turn have been backcrossed to C57BL females. The resultant backcross animals should segregate in a 50:50 ratio for the virus passed from the strain GR through the germ cells of the males if a single gene is involved. Proof that a single gene is involved will be the results of testing the offspring of these backcross animals to see if these results do indeed confirm the 50:50 ratio in the first backcross. Presence of the virus will be shown by testing for the viral antigen in the milk of each female which will be done in Dr. Parks' laboratory. Confirmation of these tests will be whether or not the females later develop a mammary tumor.

Results: We are just getting this experiment under way. Final results cannot be expected before a year and a half or two years.

Significance of the Program to the Institute: The development of the concept of genetic transmission of the oncogenic viruses is one of the most significant recent advances in cancer research. The thinking on the transmission of the mammary tumor virus led by the Dutch group is based on the idea that a single gene is involved and simple ratios tend to support this concept. However, earlier work in this laboratory and some of the results of others suggest that it may not be a single factor. The only way we can be sure is to do this breeding test of the backcross segregants so we are doing it. It will confirm or refute the present answer to the current most important question in experimental mammary cancer.

Project Title: Part II. Studies on the Mouse Mammary Tumor Virus

Principal Investigator: W. E. Heston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description: Objective 1. Approach 4

Objective: The aim of this study is to see if there is an MTV in genetically resistant strain C57BL and to see if this strain can propagate MTV introduced from susceptible strains. A second part of the study is to ascertain whether or not MTV has any influence on chemical carcinogenesis.

Methods: Several fostered lines have been started and are being followed to see if they develop mammary tumors. These include:

C57BLfRIII - the C57BL with RIII MTV to see if the MTV is propagated.

C57BLfC3H - the C57BL with C3H MTV to compare with C57BLfRIII.

BALB/cfC57BL - the C57BL with C57BL MTV if there is an MTV in C57BL. This is a test for such an MTV.

In addition, C3H and C3HfB mice have been injected with MCA to see if the presence of MTV in the C3H alters the tumor induction capacity of the MCA. Likewise, BALB/c and BALB/cfC3H mice also have been injected with MCA.

Results: A mammary tumor has occurred in the BALB/cfC57BL line in a relatively young female. It is now being examined for B particles. Further observation of the strain should ascertain whether this indicates that C57BL has MTV or this is just an unexplained chance observation.

Subcutaneous tumors are coming up in the MCA injected mice but it is too early to state whether or not they are influenced by the MTV.

Significance of Program to Institute: The study of the C57BL mice is a part of the general study of variation in host susceptibility and variation in the virus in mammary tumorigenesis in mice.

The search for an effect of MTV on MCA carcinogenesis is a part of the general study of how oncogenic viruses affect carcinogenesis with other agents. There are real effects of the C-type oncogenic viruses on chemical carcinogenesis, and there is a relationship between radiation carcinogenesis and both C and B particles, but no one has yet shown an effect of the B-type viruses on chemical carcinogenesis.

Proposed Course of Study: These fostered lines will be carried for about two more years during which time we find out whether they develop mammary tumors and with what incidences. We should have the information from the MCA injected mice within a year.

Publications:

Heston, W. E.: Genetics: Animal Tumors. In Becker, F. F. (Ed.) Cancer: A Comprehensive Treatise. New York, N.Y., Plenum Press, in press.

Serial No. NCI-3265
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mammary Tumor Viruses in the Mouse Transmitted by Milk

Previous Serial Number: Same

Principal Investigators: G. Vlahakis and W. E. Heston

Other Investigators: G. H. Smith

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description: Objective 1, Approach 4

Objectives: To compare three naturally occurring mammary tumor viruses in genetically similar susceptible hosts.

The three naturally occurring milk-borne MTV's ranged from high, to medium, to low ability in the production of mammary tumors when compared in virgin BALB/c females. This project has been completed and published in the Journal of the National Cancer Institute, Vol. 51, pages 1711-1712, 1973.

Serial No. NCI-3267
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization of DNA Methylation Activity in Subnuclear Fractions Prepared from Paired Normal and Neoplastic Mouse Cells in S Phase

Previous Serial Number: Same

Principal Investigator: David G. Longfellow, Ph.D.

Other Investigators: R. Raymond Gantt, Ph.D.
Gilbert H. Smith, Ph.D.

Cooperating Unit: Cellular Physiology and Oncogenesis Section
Laboratory of Biochemistry
National Cancer Institute

Man Years:

Total:	1-1/2
Professional:	1
Others:	1/2

Project Description:

Objective: To determine the relationship of DNA methylation to DNA replication and neoplasia.

Methods: An established mouse embryo fibroblast cell line is carried in culture as two sublines, one "normal," the other neoplastic. Neoplastic conversion is induced by supplementing the culture fluid with horse serum rather than fetal calf serum.

In studies to date, the normal subline is synchronized to the S phase of the cell cycle with Amethopterin. Prepared nuclei are disrupted by nitrogen cavitation and fractionated by discontinuous gradient techniques. The presence of DNA methylase is detected by the ability of a subfraction to transfer a labeled methyl group from S-adenosylmethionine to an endogenous or exogenous DNA acceptor. The acid washed DNA reaction products are hydrolyzed in formic acid and the base products are analyzed by high performance liquid chromatography.

Major Findings: The principal DNA methylation which we have observed with an exogenous DNA acceptor (*M. lys.*) is 5-methylcytosine. This is a result reported by many investigators as the only product of DNA methylation in eukaryotes. We have in addition, with more sensitive techniques, been able to detect a significant level of activity with another DNA base, tentatively identified as 1-methylguanine. The two enzymes, identified by their products, have different acceptor concentration requirements and storage sensitivities. Major amounts of newly synthesized DNA and DNA polymerase are found with fractions of the nucleus which contain the highest levels of these methylase activities. Results of this work have been presented at the 13th Annual Meeting of the American Society for Cell Biology and a manuscript has been submitted to *Biochemistry*.

Significance for Cancer Research: NCP Objective 3. Approach 3

These results have made it possible to now compare the DNA methylation activities of normal and neoplastic paired cell lines to determine if there is increased levels of DNA methylation associated with the neoplastic process as has been reported with RNA methylation studies. The tentative finding of 1-methylguanine is further significant in that modifications of guanine in the N-1 position would act as a stearic block to G-C base pairing and could thereby produce recognition sites for enzymes or modifiers of the DNA.

With the present system we will now attempt to synthesize new DNA *in vitro* with the DNA polymerase endogenous to the subnuclear fractions and to subsequently determine what bases of the newly synthesized DNA can be methylated *in vitro*. It is hoped that a better understanding of the effects of neoplasia on the process of DNA methylation and replication as well as a more complete understanding of the event itself will be developed as these studies are paralleled using the neoplastic subline.

Honors and Awards:

Postdoctoral Research Fellowship to David G. Longfellow, Ph.D.
Damon Runyon Memorial Fund for Cancer Research, Inc. (DRF-694)

Serial No. NCI-3268
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of Enzyme Expression in Normal Differentiating and Neoplastic Tissue

Previous Serial No.: Same

Principal Investigator: Charles K. Grieshaber, Ph.D.

Other Investigators: Harold A. Hoffman, Ph.D.
Warren H. Evans, Ph.D.

Cooperating Unit: Laboratory of Biochemistry, NCI

Man Years:

Total:	3
Professional:	1-1/2
Others:	1-1/2

Project Description:

Objectives: To study the appearance or loss of enzyme activities in normal developing tissues and tumors in order to define some of the processes which regulate cellular differentiation and subsequent deviations from normal molecular events. Similarities and differences between these processes which regulate enzyme activities in normal differentiating and non-differentiating cells and neoplastic cells is essential to this understanding.

Methods: Tissues such as mammary gland and mammary tumors, liver and hepatomas, kidney, and bone marrow and peripheral blood cells are used as the source of enzymes upon which appropriate physical, biochemical, genetic, and immunochemical analyses are carried out. The enzymes currently under investigation are catalase, peroxidase, arginase and adenylcyclase--those enzymes in which regulatory factors such as hormones can influence modulation. Mice from eight inbred strains and strain 13 guinea pigs are used as the source of tissues.

Major Findings:

1) Erythrocyte catalase has been isolated from BALB/c mice and humans. The two enzymes appear to be indistinguishable by immunochemical methods; the human preparation has a specific activity of 6.8×10^7 M^{-1} sec^{-1} whereas the murine enzyme has a specific activity of 2.7×10^7 M^{-1} sec^{-1}. Rabbit anti BALB/c RBC catalase cross reacts with erythrocyte, liver and kidney catalase from several other strains of mice. In an effort to describe the mechanism responsible for differing electrophoretic mobilities, C57BL/6 and C3Hf kidney catalases have been isolated. The C3Hf kidney catalase appears to be bound to a lipid containing molecule while that from C57BL/6 kidney is not bound. There is little if any difference in their respective specific activities (2.3×10^7 cf 2.1×10^7). Immunoprecipitation curves are currently being set up and analyzed for quantitative detection of catalase concentrations in tissues from BALB/c, C57BL/6, C57BL/An, NBL, and C3Hf strains of mice.

2) Studies on the catalase activity in C3H-A^{vy} mice bearing hepatomas and normal liver showed that the normal tissue: tumor activity is 1.9-2.2.

3. Arginase levels were investigated in bone marrow cells from BALB/c, C57BL/6, AKR, C57BL/An, DBA/2e, and GR strains of mice. Little or no differences were observed in the arginase content of the different strains. 3',5' cyclic AMP (cAMP) levels were analyzed in guinea pig bone marrow cells to determine the effect of hormones on cell maturation. The cyclic AMP levels were found to be proportional to the degree of maturation of the cells, with blast containing approximately 2 pmole cAMP 10^7 cells and mature PMN's containing 4-6 pmole cAMP per 10^7 cells.

Significance for Cancer Research: NCP Objective 3. Approach 3

This project provides a model system in tissues from animals of known genetic composition for the study of protein and enzyme regulation and interaction during neoplastic transformation. This system may have wide significance to general processes of differentiation and tumorigenesis since understanding the several potential points in the regulation of these enzymes will provide a description of metabolic sequences and biochemical lesions.

Proposed Course:

1) Analysis of kidney, RBC, WBC, and mammary tissue catalase content will be completed using our existing immunochemical reagents.

2) Mammary glands and tumors in various stages of development will be characterized in terms of mixed oxidase enzymes and catalase as well as in terms of their responsiveness and regulation by prolactin.

3) The rate of synthesis and degradation of catalase will be studied in mammary glands of GR, C3HfB, C3H, C3H-A^{vy}, DBA/2, BALB/c and C57BL/6 mice in an effort to understand the mechanisms involved in elevated catalase activity in mammary tumorigenesis.

4) Bone marrow cells from normal guinea pigs and inbred mice and leukemic mice will be cultured in order to study the hormonal involvement during normal and abnormal differentiation.

Publications:

Grieshaber, C. K. and Hoffman, H. A.: A rapid and convenient method for isolation of murine erythrocyte catalase by DEAE-cellulose chromatography and gel filtration. Anal. Biochem., in press.

Serial No. NCI- 3269
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Etiology of Carcinomatous Ascites

Previous Serial Number: None

Principal Investigator: George B. Feldman, M.D.

Other Investigators: None

Man Years:

Total: 1
Professional:
Others:

Project Description:

Objectives:

1. Determine the role of lymphatic obstruction in the etiology of a murine carcinomatous ascites.

2. Determine the influence of anti-coagulants and fibrinolytics on the development of a murine carcinomatous ascites.

Methods:

1. Histological: Step-serial sections of *en bloc* mouse abdomen and thorax.

Major Findings:

1. Murine ovarian cancer cells do not enter the lymphatic channels draining the peritoneal cavity prior to the formation of ascites.

2. Blockage of lymphatic drainage seems to occur as a result of the layering out of tumor cells over the draining surface of the peritoneum.

3. Neither systemic heparin, systemic coumarin, nor intraperitoneal heparin inhibits the formation of murine ovarian carcinomatous ascites.

Honors and Awards: --

Publications:

Feldman, G. B., and Knapp, R. C.: Lymphatic drainage of the peritoneal cavity and its significance in ovarian cancer. Accepted for publication in the *American Journal of Obstetrics and Gynecology*.

Significance for Cancer Research: Supporting Objective 4-2.

This project suggests the value of a shift in perspective regarding the dissemination of ovarian cancer, and, consequently, a shift in emphasis in therapy.

Proposed Course: Finalize data and terminate project by the end of June, 1974.

Serial No. NCI-3270
1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Host Genome-Viral Relationship in Mammary Tumor Development in Mice

Previous Serial Number : None

Principal Investigator: G. Vlahakis

Other Investigators: W. E. Heston and G. H. Smith

Man Years:

Total: 3/4
Professional: 1/2
Other: 1/4

Project Description: Objective 1, Approach 4

Objectives: To study the relationship between genetic factors and the milk-borne viral influence (MTV) in mammary tumorigenesis.

Methods: Strain C3HfBfDD (with DD-MTV) when kept as virgins, has an approximate 72% mammary tumor incidence occurring at 15.2 months of age. The comparable strain C3H-A^{vy}fB (with the A^{vy} gene) was foster nursed on strain DD to produce C3H-A^{vy}fBfD (with DD-MTV). Our purpose in developing this fostered strain is to determine if the DD-MTV in the presence of the A^{vy} gene will increase mammary tumors in C3H-A^{vy}fBfDD significantly over that in virgin C3HfBfDD females, and at the same time reduce tumor age.

There are now a group of approximately 100 C3H-A^{vy}fBfDD females being maintained as virgins, and a similar number as breeders to compare with C3HfBfDD breeders. Both breeder and virgin C3H-A^{vy}fBfDD females are presently being examined for mammary tumors.

Results: Thus far, 12 C3H-A^{vy}fBfDD breeders have developed mammary tumors at an average tumor age of 8.3 months, which compares well with the comparable strain C3HfBfDD breeders. Four of the C3H-A^{vy}fBfDD virgins had mammary tumors at an average tumor age of 9.0 months, which is significantly less than the 15.2 months for the C3HfBfDD virgin females.

These early results suggest that strain C3H-A^{vy}fB virgins may be as susceptible to mammary tumors with the less potent DD-MTV as C3HfBfC3H virgins are with the more virulent C3H-MTV (9.6 months, tumor age). In the virgin C3H-A^{vy}fBfDD females tumor age appears to be markedly reduced, and the final incidence compared to that for C3HfBfDD virgins (72%) may approach 100%. We will have more information later this year.

Methods: Substrain C57BL/He which is one of the most resistant mammary tumor strains, is genetically resistant to the virulent strain C3H mammary tumor virus (MTV), losing it in a generation or two after its introduction. The genetically similar substrain C57BL/M of Dr. Dan H. Moore at the Institute for Medical Research, Camden, New Jersey, has been shown to be more susceptible than C57BL/He to the development of both spontaneous and induced mammary tumors. We have now developed C57BL/MfC3H (with C3H-MTV) and hope to determine how well C3H-MTV survives in this fostered strain.

There are approximately 148 females being normally maintained as breeders and upon completion of their reproductive period, they are being placed in retired cages and are being regularly examined for mammary tumors.

Results: In one line, a C57BL/MfC3H female was necropsied at 13 months of age with a mammary tumor. Histologic examination of this tumor has shown it to be an adenocarcinoma type B. When the tumor was examined by the electron microscope, many B particles were observed. A sister to this female with the mammary tumor was necropsied at 19 months without a tumor. Later this year we hope to have more information on C57BL/MfC3H.

Methods: Interestingly, Dr. Moore has reported on the high incidence of mammary tumors in strain C57BL/M when given MTV from strain RIII. Since strains RIII and DD are of European origin, and both develop hormone dependent mammary gland lesions, and therefore they may be related, we have now developed the fostered strain C57BL/MfDD (with DD-MTV). If the two strains are related, and presumably their MTV's as well, then we would expect to see a similar high incidence of mammary tumors in C57BL/MfDD. We are now in the process of mating all of the animals and hope to have approximately 100 females in this study.

Methods: To compare the GR-MTV from the high European mammary tumor strain GR, with C3H-, A-, and DD-MTV's, we are developing the fostered strain BALB/cfGR (with GR-MTV). The BALB/c females are susceptible to mammary tumors when given MTV through foster nursing on strains that have the milk-borne virus. BALB/cfGR females will be maintained as virgins to make possible a comparison with the earlier study, in which it was shown that the three naturally occurring MTV's from strains C3H, A, and DD ranged from high, to medium, to low ability in the production of mammary tumors only when compared in virgin BALB/c females.

Methods: In 1971 Hageman and Calafat of the Netherlands Cancer Institute, reported finding occasionally mammary tumor virus (MTV) antigens in mammary glands of old uninfected breeding females of strain BALB/c. This strain is widely distributed and is known as a low spontaneous mammary tumor strain. When cell-free homogenates or preparations from old mammary glands were injected into young BALB/c females, they caused a high percentage of mammary tumors at six months of age. This they believed was evidence that strain BALB/c has MTV. We have attempted to repeat their work with our strain BALB/c, for which we have no evidence that it has MTV.

Results: Sixty-seven young BALB/c females received intraperitoneal injections of cell-free extracts of mammary glands from old uninfected breeding females of our strain BALB/c. Not a single mammary tumor occurred in the females necropsied at an average time of 13.6 months, from the time of injection to the time of death, and the average age at death was 15 months.

Cell-free extracts were also prepared from two old BALB/c mammary tumor transplants, one designated as 9436, and the other 20143, these being the numbers of the original females in which the tumors arose spontaneously. The two transplants have always been carried in strain BALB/c. No mammary tumors occurred in 18 BALB/c females that had received intraperitoneal injections of cell-free extracts from BALB/c mammary tumor transplant 9436. They were necropsied at an average time of 16.5 months, from the time of injection to the time of death, and the average age at death was 18.3 months.

Similarly, no tumors occurred in 24 BALB/c females that received injections of cell-free extracts from BALB/c mammary tumor transplant 20143. They were necropsied at an average time of 15.4 months, and the average age at death was 17.4 months.

Our BALB/c colony continues to be a low spontaneous mammary tumor strain, and the results with cell-free extracts of old mammary glands and tumor transplants support the view that BALB/c does not have a mammary tumor virus.

Sabine, Horton, and Wicks in 1973, at Waite Agricultural Research Institute at the University of Adelaide, in Adelaide, S.A., Australia, attributed the high spontaneous mammary and liver cancer in our strains C3H-A^{vy} and C3H-A^{vy}fB in the United States to the use of cedar shavings in the bedding material. Although this conclusion was based on small numbers of animals, they considered cedar to be the carcinogenic agent. Since their publication we have been setting up some matings of strain C3H-A^{vy}fB on pine bedding material without the cedar. Upon completion of the reproductive period, approximately 50 females will be retired 8 to a cage, continually maintained on pine and regularly examined for the development of tumors.

It is hoped that this year in collaboration with Dr. Melvin Reuber of the University of Maryland Medical School, we will be submitting a manuscript to the JNCI on the spontaneous hyperplastic and neoplastic lesions of the uterus that occur in our female mice. Such a publication we believe should prove useful to those investigators who are working with these tumors in mice and possibly other rodents.

During this year, opportunities both here and outside the NIH have arisen for mammary tumor and hepatoma studies with other investigators. We believe our contribution of valuable mammary tumor and hepatoma mouse strains, and our knowledge of the biology of these neoplasms make such collaborative studies desirable, and therefore, several of these are now being actively pursued. Also some time has been spent during this past year with research workers both here and outside of the NIH, helping them to develop their own programs. This has consisted of discussions and of making available to them specific biological material for their research requirements.

Significance of Program to the Institute:

The occurrence of mammary tumors in mice depend on the interplay of three factors: the genotype of the animal, hormones, and the mammary tumor virus (MTV). The A^{vy} gene of strain C3H-A^{vy}fB is an example of the role of genes in tumorigenesis. Tumors result from the interaction of a suitable genotype with hormones and viral agents. The viable yellow gene (A^{vy}) raises the mammary tumor incidence from approximately 40% in strain C3HfB breeders to approximately 90% in the comparable strain C3H-A^{vy}fB breeders, and reduces the average tumor age from 19 months in C3HfB to 15 months in C3H-A^{vy}fB. When maintained as virgins, C3H-A^{vy}fB has a mammary tumor incidence of 20%, compared to approximately 10% for C3HfB.

The DD-MTV which has been shown to be a low oncogenic virus when compared with C3H- and A-MTV's in genetically similar susceptible BALB/c virgin females, is now being tested in C3H-A^{vy}fB to study the host genome-viral relationship in mammary tumor development in this strain. We believe similar studies with the different milk-borne MTV's and the genetically variable mouse strains, will help in assessing the relative importance of these factors in mammary tumorigenesis.

Proposed Course of Experiment:

The study of foster-nursed strain C3H-A^{vy}fBfDD (with DD-MTV) is now well under way and should be nearly complete by the end of this year.

Publications:

Vlahakis, G.: Comparison of three mammary tumor viruses in genetically similar susceptible mice. J. Nat. Cancer Inst. 51: 1711-1712, 1973.

Woods, Mark W., and Vlahakis, G.: Immediate effects of insulin and anti-insulins on glycolysis in spontaneous mammary tumors in mice. J. Nat. Cancer Inst. 52: 579-582, 1974.

SUMMARY STATEMENT
LABORATORY OF MOLECULAR BIOLOGY
DCBD, NCI
July 1, 1973 - June 30, 1974

Research in the Laboratory of Molecular Biology continues to be centered in three areas.

1. What is the role of cyclic AMP in malignant transformation?

2. How is gene activity controlled?

3. What is the mechanism of genetic recombination?

Cyclic AMP and Malignant Transformation--Over the last few years we showed that transformed embryonic fibroblasts had low levels of cyclic AMP and presented evidence that the decreased adhesiveness, rounded shape, rapid growth and increased agglutinability of transformed cells was due to their low levels of cyclic AMP. M. Willingham has now strengthened the cause and effect relationship between cyclic AMP and these properties by isolating a mutant of mouse 3T3 cells in which cyclic AMP levels can be decreased by a fall in temperature. Shortly after cyclic AMP levels fall the untransformed cells decrease their adhesiveness, retract their processes, round up and become very agglutinable. Prior exposure of the cells to Bt_2cAMP blocks these responses.

We believe cyclic AMP may control cell shape, adhesiveness and agglutinability by modulating the behavior of the contractile protein myosin. R. Ostlund has prepared large amounts of myosin from fibroblasts and made antibodies to this myosin. Using immunological methods, M. Willingham has shown some of the myosin to be located in the plasma membrane of intact cells, a location where the protein can affect many aspects of cell behavior.

W. Anderson has been examining the reason cyclic AMP levels are low in transformed cells. In all cases studied the enzyme that makes cyclic AMP, adenylate cyclase, has decreased activity. It is particularly interesting that each different "transforming" virus inactivates adenylate cyclase by a different mechanism.

Transformed cells have altered energy metabolism with increased glycolysis. To date we have found no role for cyclic AMP in controlling glycolysis, but G. Johnson with J. Schwartz and J. Passonneau of NICHD have found that transformed cells have a low ratio of NAD^+/NADH. We are now trying to understand what relationship exists between cyclic AMP, NAD^+ and NADH.

Control of Gene Activity--These studies have so far been conducted in bacteria and bacterial viruses, though studies with animal cells may be initiated this next year.

S. Nakanishi has continued to investigate how cyclic AMP and the cyclic AMP receptor (CRP) regulate initiation of transcription. All our evidence is consistent with cyclic AMP and CRP altering the structure of DNA to allow RNA polymerase to initiate transcription. To understand how this occurs, we are determining the structure of CRP and the structure of the DNA where CRP binds. In collaboration with H. Niall at Harvard Medical School we have now determined about one third of the structure of CRP. Studies conducted by B. de Crombrugghe, R. Musso and S. Weissman (Yale Medical School) may soon allow us to determine the structure of the CRP binding site. So far the sequence of the first 75 bases of gal operon RNA made in vitro has been determined. (From this the sequence of the DNA can be inferred.) If this sequence does not include the CRP binding site (or the binding site of the gal repressor), the sequence will be extended to ascertain these.

B. de Crombrugghe and M. Rosenberg (Yale) are also determining the structure of DNA at sites where RNA transcription stops. Soon we will know what the signals are in bacterial DNA that begin and terminate transcription.

S. Adhya, B. de Crombrugghe and M. Gottesman have found that when the bacterial virus (λ) is integrated into the bacterial chromosome, transcription beginning in a λ gene will extend into nearby bacterial genes. This occurs because the virus makes a protein which tells RNA polymerase to ignore the usual transcriptional stop signals. The bacterial mRNA made in this manner is much more stable and therefore more efficiently translated into protein than bacterial messenger RNA made in the usual manner. These experiments have important implications for how animal viruses might "transform" animal cells.

Genetic Recombination--The biochemical basis of genetic recombination is unknown. To study it M. Gottesman and M. Shulman have isolated two types of mutants of λ. One class of these has a segment of the E. coli chromosome attached to λ in such a manner that it can be excised by a normal excision step. The other class has defective attachment sites so that recombination is impaired. These mutant DNAs are the substrates for in vitro recombination experiments now in progress.

Serial No. NCI-8000
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of Gene Expression in *E. Coli* by Cyclic AMP

Previous Serial Number: Same

Principal Investigator: Ira H. Pastan

Other Investigators: Wayne B. Anderson, Shigetada Nakanishi, and Mordechai Aboud

Cooperating Units: None

Man Years: July 1, 1973 through June 30, 1974

Total:	3.3
Professional:	2.8
Others:	0.5

Project Description:

Objectives: Cyclic AMP is required for the synthesis of a variety of enzymes in *E. coli*, most of which are inducible. Cyclic AMP acts at a transcriptional level. Our objective is to determine how cyclic AMP, cyclic AMP receptor protein (CRP) and other factors control gene activity.

Methods Employed: Studies on mRNA and enzyme synthesis have been carried out in intact cells and in cell-free extracts. Mutants which are defective in cyclic AMP metabolism have been isolated and characterized. Proteins controlling gene transcription are isolated and their mechanism of action studied.

Major Findings: We previously have shown that cyclic AMP and cyclic AMP receptor (CRP) are necessary to initiate transcription of the *lac*, *gal* and other operons. We have devised a new method of preparing CRP in sufficient quantitites to perform physical studies on the molecule. In collaboration with Drs. Hugh Niall and David Schlessinger at Harvard Medical School we have now determined about 50% of the amino acid sequence of the protein. With Dr. Joseph Krakow of Hunter College we have obtained a fragment of CRP that binds to DNA in the absence of cyclic AMP. This fragment contains the N-terminal portion of CRP.

S. Nakanishi has been studying how CRP works and finds that agents that "melt" DNA such as glycerol can eliminate the requirement for cyclic AMP and CRP both *in vitro* and *in vivo*. This finding strengthens the conclusion from other data that cyclic AMP and CRP act by modifying DNA so that RNA polymerase can bind and initiate transcription.

M. Aboud has identified and purified a protein from E. coli that is needed for another small nucleotide (ppGpp) to initiate lac transcription. This protein and ppGpp are only active in the presence of cyclic AMP and CRP.

Significance to Biomedical Research and the Program of the Institute: In cancer cells, genes which are normally quiet become active due to some alteration in the control of gene expression. It is not yet possible to study the expression of individual genes in animal cells. Therefore, our studies on the control of the transcription of lac and gal genes by cyclic AMP, cyclic AMP receptor protein, lac repressor, gal repressor and ppGpp are a basis on which to build an understanding of how gene expression is controlled in higher organisms.

Proposed Course: To examine further the mechanism by which cyclic AMP, CRP and other factors instruct RNA polymerase to initiate gene transcription.

Honors and Awards: None

Publications:

Nakanishi, S., Adhya, S., Gottesman, M. and Pastan, I.: Studies on the Mechanism of Action of the Gal Repressor. J. Biol. Chem. 248: 5937-5942, 1973.

De Crombrugghe, B. and Pastan, I.: Regulation of Gene Expression by Cyclic AMP in Escherichia Coli. In Davies, D.D. (ed.): Rate Control of Biological Processes. Cambridge, England, University Press, 1973, pp. 365-369.

Anderson, W.B. and Pastan, I.: The Cyclic AMP Receptor of E. Coli: Immunological Studies in Extracts of E. Coli and Other Organisms. Biochim. Biophys. Acta 320: 577-587, 1973.

Krakow, J.S. and Pastan, I.: Cyclic Adenosine Monophosphate Receptor: Loss of cAMP Dependent DNA Binding Activity after Proteolysis in the Presence of cAMP. Proc. Nat. Acad. Sci. U.S.A. 70: 2529-2533, 1973.

Pastan, I., Gallo, M. and Anderson, W.B.: The Purification and Analysis of Mechanism of Action of a Cyclic AMP-Receptor Protein from E. Coli. In Methods in Enzymology, in press, 1974.

Anderson, W.B., Gottesman, M.E. and Pastan, I.: Studies with the Cyclic Adenosine Monophosphate Receptor and Stimulation of In Vitro Transcription of the Gal Operon. J. Biol. Chem., in press, 1974.

Nakanishi, S., Adhya, S., Gottesman, M. and Pastan, I.: Activation of Transcription at Specific Promoters by Glycerol. J. Biol. Chem., in press, 1974.

Serial No. NCI-8001
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed and Normal Fibroblasts

Previous Serial Number: Same

Principal Investigator: Ira Pastan

Other Investigators: Wayne Anderson, Mark Willingham, Richard Carchman, Tom Russell, Rich Ostlund and Robert Adelstein

Cooperating Units: Cardiology Branch, National Heart and Lung Institute

Man Years: July 1, 1973 through June 30, 1974

Total:	6.1
Professional:	5.1
Others:	1.0

Project Description:

Objective: To investigate how cyclic AMP regulates the growth and morphology of normal and transformed fibroblasts, and to determine the nature of the alteration of cyclic AMP metabolism in transformed fibroblasts.

Methods Employed: Cell culture and standard biochemical analyses of the enzymes involved in cyclic AMP metabolism and the synthesis of macromolecules.

Major Findings: The past year has been devoted to trying to understand why cyclic AMP levels low in transformed cells become elevated in normal cells and how cyclic AMP controls cell shape, adhesiveness and agglutinability.

How do normal cells elevate their cyclic AMP levels at confluency? In growing cells the activity of both a.c. and cyclic AMP phosphodiesterase (PDE) is low, but both increase as the cells grow together. When the cells become quite crowded the PDE stops rising, the a.c. continues to rise and cyclic AMP levels go up.

Generally, in transformed cells both a.c. and PDE are diminished. Different viruses decrease adenylate cyclase by diverse mechanisms. In chick embryo fibroblasts (CEF) transformed by RSV-BH, a.c. has an altered k_m (ATP), whereas CEF transformed by RSV-SR have a decreased amount of a soluble activator of the enzyme. In NRK cells transformed by Kirsten and Moloney viruses the k_m (ATP) is normal but the enzyme cannot be activated by either the soluble activator or Mg^{++}. We interpret these results to mean that different viruses have different "transforming" genes.

Although increased PDE would be one way to lower cyclic AMP levels, we have not yet found a transformed cell in which this occurs. Indeed PDE is induced by cyclic AMP. There are at least two forms of PDE in cells; only one of these is inducible. This enzyme has a very short half-life, about two hours.

To study how cAMP controls agglutination M. Willingham devised a quantitative way to study this using the cell-sizing feature of a cell counter. We find that there is a very good correlation between cyclic AMP levels and agglutinability of both normal and transformed cells. This greatly strengthens the idea that the increased agglutinability of transformed cells is due to their low cAMP levels.

Marcomolecules that are good candidates as participants in mediating changes in cell motility, shape, and agglutinability are myosin, actin, and tubulin. With R. Adelstein (NHLI) we found that fibroblasts contained myosin. R. Ostlund has purified large amounts of this myosin and prepared goat antibodies to it. These antimyosin antibodies agglutinate L cells and give membrane fluorescence in the presence of fluorescein labelled antigoat gamma globulin. We conclude that myosin must insert into the plasma membrane, a finding consistent with its proposed role in mediating cell motility, adhesiveness and agglutinability.

M. Willingham has isolated a mutant of untransformed 3T3 cells whose cyclic AMP levels fall, when the cell is exposed to temperature change. Following temperature shift the sequence of events is as follows: 0-2 min, cAMP levels fall by 50%; 2-4 min, cell adhesiveness decreased; 4-10 min, cellular processes retracts, cells round up and detach from dish and become very agglutinable. Treatment of these cells with Bt_2cAMP before temperature shift prevents all these responses. These studies indicate that cyclic AMP controls the morphology, adhesiveness, and agglutinability of normal 3T3 cells.

Significance to Biomedical Research and the Program of the Institute: Part of the reason for the abnormal behavior of transformed cells in culture is their inability to maintain normal cyclic AMP levels. If one could raise levels in these cells, one might control the growth of certain types of cancers.

Proposed Course: 1. We will examine how the transformation event alters the ability of cells to maintain normal cyclic AMP levels. We also will study how cyclic AMP works to slow growth and produce its other effects.

2. We will investigate why some cells are much less responsive to cylcic AMP than others.

Honors and Awards: DHEW Superior Service Award, June 11, 1973
N.I.H. G. Burroughs Mider Lectureship, Nov. 7, 1973

Publications:

Rein, A., Carchman, R., Johnson, G.S. and Pastan, I.: Simian Virus 40 Rapidly Lowers cAMP Levels in Mouse Cells. Biochem. Biophys. Res. Commun. 52: 899-904, 1973.

Anderson, W.B., Lovelace, E. and Pastan, I.: Adenylate Cyclase Activity is Decreased in Chick Embryo Fibroblasts Transformed by Wild Type and Temperature Sensitive Schmidt-Ruppin Rous Sarcoma Virus. Biochem. Biophys. Res. Commun. 52: 1293-1299, 1973.

Russell, T. and Pastan, I.: Plasma Membrane Cyclic Adenosine 3':5'-Monophosphate Phosphodiesterase of Cultured Cells and its Modification after Trypsin Treatment of Intact Cells. J. Biol. Chem. 248: 5835-5840, 1973.

Pastan, I., Willingham, M., Carchman, R. and Anderson, W.B.: Cyclic AMP Metabolism in Normal and Transformed Fibroblasts. In The Role of Cyclic Nucleotides in Carcinogenesis--Miami Winter Symposia. New York, N.Y., Academic Press, 1973, Vol. 6, pp. 47-56.

Willingham, M. and Pastan, I.: A Mutant of 3T3 Cells with Cyclic AMP Metabolism Sensitive to Temperature Change. Proc. Nat. Acad. Sci. U.S.A. 70: 2906-2910, 1973.

Anderson, W.B., Russell, T.R., Carchman, R.A. and Pastan, I.: Interrelationship Between Adenylate Cyclase Activity, Adenosine 3':5' Cyclic Monophosphate Phosphodiesterase Activity, Adenosine 3':5' Cyclic Monophosphate Levels, and Growth of Cells in Culture. Proc. Nat. Acad. Sci. U.S.A. 70: 3802-3805, 1973.

Carchman, R.A., Johnson, G.S., Pastan, I. and Scolnick, E.M.: Studies on the Levels of Cyclic AMP in Cells Transformed by Wild-Type and Temperature-Sensitive Kirsten Sarcoma Virus. Cell 1: 59-64, 1974.

Pastan, I., Anderson, W.B., Carchman, R.A., Willingham, M.C., Russell, T.R. and Johnson, G.S.: Cyclic AMP and Malignant Transformation. In B. Clarkson and R. Baserga (eds.): Control of Proliferation in Animal Cells. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, in press, 1974.

Pastan, I. and Johnson, G.S.: Cyclic AMP and the Transformation of Fibroblasts. In Advances in Cancer Research, in press, 1974.

Ostlund, R.E., Pastan, I. and Adelstein, R.: Myosin in Cultured Fibroblasts. J. Biol. Chem., in press, 1974.

Schwartz, J.P., Johnson, G.S., Pastan, I. and Passonneau, J.V.: The Effect of Growth Conditions on NAD^+ and NADH Concentrations and the NAD^+/NADH Ratio in Normal and Transformed Fibroblasts. J. Biol. Chem. in press, 1974.

Serial No. NCI-8005
1. Laboratory of Molecular Biology
2. Section of Biochemical Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Biochemistry of λ Int-Promoted Recombination

Previous Serial Number: Same

Principal Investigators: Susan Gottesman, Marc Shulman and Max E. Gottesman

Other Investigator: None

Cooperating Unit: None

Man Years: July 1, 1974 through June 30, 1974

Total:	2.7
Professional:	2.7
Others:	0

Project Description:

Objectives: Phage-induced functions promote the integration of λ DNA into the *E. coli* genome. Our objective is to study these functions at the genetic and biochemical level.

Methods Employed: Standard microbiological techniques and techniques developed for this project were used in this study.

Major Findings: (a) We have isolated λ with mutant attachment sites. The mutations reside in a region of homology between λ and *E. coli*.

(b) The functions which promote integration cannot work sequentially. Translation, transcription, and replication are not required for the reaction.

Significance to Biomedical Research and the Program of the Institute: The biochemical basis of phage integration is at present completely unknown. The phage integration reaction will serve as a model system for studying the integration of oncogenic viruses in mammalian cells.

It provides information of potential value in determining the nature of oncogenic cell transformation and thus may ultimately permit the prevention of conversion of normal to tumorigenic cells.

Proposed Course: We have now developed a system which permits us to begin to examine the biochemical basis of λ integration. We hope to use this system to detect the activity of phage integration functions and to elucidate the nature of the reaction they catalyze. We plan to isolate new λ mutants with integration-excision defects.

Honors and Awards: None

Publications:

Shulman, M., and Gottesman, M.E.: Attachment Site Mutants of Bacteriophage λ. J. Mol. Biol. 81: 461-483, 1974.

Gottesman, M.E.: The Integration and Excision of Bacteriophage Lambda. Cell 1: 69-72.

Serial No. NCI-8006
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Control of Expression of the Galactose Operon in E. coli K12

Previous Serial Number: Same

Principal Investigator: Sankar L. Adhya

Other Investigators: Max E. Gottesman and Benoit de Crombrugghe

Cooperating Units: None

Man Years: July 1, 1973 through June 30, 1974

Total:	1.3
Professional:	1.3
Others:	0

Project Description:

Objectives: The enzymes of the galactose operon of E. coli are needed for utilization of galactose and normal cell morphology. The control of expression of the operon is complex. It is normally subjected to a negative control by gal repressor and a positive control by cyclic AMP. We have also shown that the galactose operon is and can be also subject to other control systems, including control by neighboring prophage genes. Our object is to determine the complete structure and to identify all the regulatory elements of the galactose operon of E. coli.

Methods Employed: Standard microbial genetic and biochemical techniques.

Major Findings: The galactose operon is located close to the prophage λ on the chromosome of E. coli. It is known that induction of the prophage causes constitutive expression of the operon (escape synthesis). Last year we reported that one of the components of the escape synthesis is due to transcription originating in the prophage DNA and extending into gal operon. We have further shown that this phage promoted transcription is (i) not blocked by galactose repressor and (ii) not subject to normal cellular transcription termination mechanisms. The requirements of such termination resistant transcription have been defined.

Significance to Biomedical Research and the Program of the Institute: In cancer cells, the expression of some genes are permanently turned on, i.e. expressed constitutively. Our studies are aimed to understand the molecular basis of how genes are turned on and off. We are using *gal* as a model system. This understanding might help to prevent conversions of cells to those capable of forming cancers.

Proposed Course: 1. To determine the complete structure of the *gal* operon. 2. To identify all the regulatory molecules involved in turning on and off of the various genes of the *gal* operon.

Honors and Awards: None

Publications:

Adhya, S., Gottesman, M., and de Crombrugghe, B.: Release of Polarity in *E. coli* by gene *N* of Phage Lambda: Termination and Antitermination of Transcription. *Proc. Nat. Acad. Sci. U.S.A.*, 1973, in press.

Serial No. NCI-8007
1. Laboratory of Molecular Biology
2. Section of Biochemical Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Positive and Negative Regulations by Bacteriophage Lambda Gene Products

Previous Serial Number: Same

Principal Investigators: Donald L. Court and Max E. Gottesman

Other Investigators: None

Cooperating Units: None

Man Years: July 1, 1973 through June 30, 1974

Total:	1.3
Professional:	1.3
Others:	0

Project Description:

Objectives: We would like to define the events necessary for the establishment of lysogeny in a λ-infected cell. To do this, we have studied the cII and cIII functions of λ by genetic and biochemical approaches. These two λ functions are both known to be required for efficient λ lysogeny and λcI-repressor synthesis.

We are also studying the λ N gene function of λ which is required as a primary activator for all other λ functions including cII and cIII.

Methods Employed: Standard microbiological and biochemical techniques have been used in these studies.

Major Findings: cII and cIII mutants have been found to be deficient in the synthesis of cI repressor protein following infection and also to overproduce lytic gene proteins. cY mutants show this same phenotype but have been shown not to be mutant in a diffusible protein (like cII or cIII) but rather in a site. The action of cII and cIII function is proportional to the multiplicity of infecting $cII^+ cIII^+$ phage.

A protein activity has been found in extracts of λcI^- infected cells that is able to bind specifically ^{32}P labelled λDNA to a membrane filter. Mutations in cII and cIII remove this activity.

SDS-acrylamido gel analysis using double labeled proteins reveals that ∿45,000 and ∿35,000 molecular weight peptides are lost when λ is cII^-. These peptides are associated with the ribosomes and eluted with 0.5M NH_4Cl.

Significance to Biomedical Research and the Program of the Institute: As suggested recently by Huebner and Todaro (P.N.A.S. 64: 1087, 1969) a process analogous to lysogenization may be important in carcinogenesis. The relatively simple phage λ is a perfect model to use for an *in vitro* study of this process. As yet little is actually known about the regulation involved at the biochemical level for even the λ lysogenization system.

Proposed Course: We are using an *in vitro* protein synthesizing system (an *E. coli* S30 system) to purify and characterize λ's *N* function and the cII and cIII functions. λ phage have been isolated through genetic crosses that contain appropriate DNA markers (mutations) for use as templates in this S30 system.

Honors and Awards: None

Publications: None

Serial No. NCI-8008
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Initiation and Termination of Transcription of Bacterial Operons

Previous Serial Number: None

Principal Investigator: Benoit de Crombrugghe

Other Investigators: Richard Musso, Sankar Adhya, Max E. Gottesman, and Ira Pastan

Cooperating Units: None

Man Years: July 1, 1973 through June 30, 1974

Total:	2.5
Professional:	2.2
Others	0.3

Project Description:

Objective: To understand the precise biochemical mechanisms which control initiation and termination of transcription in *E. coli*.

Methods Employed: Measurement of RNA and protein synthesis *in vivo* and in cell-free systems; isolation of factors active in regulation of initiation and termination of transcription; purification of specific RNA species; nucleotide sequence analysis of RNA.

Major Findings: (a) Transcription of the *lac* and *gal* operons of *E. coli* is known to be regulated by cyclic AMP, a pleiotropic protein, the cyclic AMP receptor, and by operon specific repressor-inducers systems. In addition, other factors are probably involved in controlling the expression of these operons. Using our previously developed purified cell-free system for synthesis of *gal* and *lac* mRNA, we have worked out methods for the synthesis and purification of a short RNA molecule suitable for sequence analysis. The nucleotide sequence of a RNA molecule 76 nucleotides long, corresponding to the 5' end of *gal* mRNA, has been determined. The sequence is:

$$\text{pppA-U-A}\ \boxed{\text{C-C-A-U}}\ \text{-A-}\boxed{\text{A-G}}\text{-C-}\boxed{\text{C-U}}\text{-A-}\boxed{\text{A-U-G-G-}}\text{-A-G-}$$

10 20

$$\text{C-}\underline{\text{G}}\text{-}\underline{\text{A}}\text{-}\underline{\text{A}}\text{-}\underline{\text{U}}\text{-}\underline{\text{U}}\text{-A-}\underline{\text{U}}\text{-}\underline{\text{G}}\text{-}\underline{\text{A}}\text{-}\underline{\text{G}}\text{-A-}\underline{\text{G}}\text{-U-U-C-U-G-G-U-U-A-C-C-G-G-U-G-}$$

30 40

$$\text{U-A-G-C-G-}\boxed{\text{G}}\text{-U-U-A-C-A-U-U-G-G-A-A-G-U-C-A-U-A-C-C-U-G-U}$$

50 60 70

The sequence of residues 4-18 reflects a partial two-fold symmetry in the DNA centered at position 11. Twelve of these fifteen nucleotides can be base paired (indicated by boxes). The sequence of residues 22-33 shows a close homology (10 of 12 bases identical as underlined) with part of the lac operator sequence (Gilbert and Maxam, PNAS, 1973). Residues 37 to 59 form another partial symmetry. Present studies aim at extending this sequence and defining the various regulatory segments on the DNA.

(b) The nucleotide sequence at the 3' end of the 6S λRNA and the 4S λRNA have been determined. Transcription of the 6S RNA terminates spontaneously in the absence of rho. Termination of transcription of the 4S RNA species is enhanced by rho. The sequence at the 3' end of λ6S RNA is mainly AU_6A (82%) and AU_6AU (6%). Several other related sequences AU_6, AU_6A_2, AU_6A_3, AU_6A_4 are also present at the 3' end of a small proportion of the 6S RNA species. Addition of rho increases the proportion of molecules terminating in AU_6AU. The majority of the 4S RNA molecules terminates with the sequence AU_6A (80%) and U_6AU (12%). A small proportion of 4S molecules also terminates in U_6A_2, U_6A_3, U_6A_4, U_6AUA. Addition of rho greatly enhances the proportion of molecules terminating in U_6AU, and U_6AUA. Current studies are directed toward determining the sequences of the DNA throughout the termination region of the 4S gene as well as the intercistronic region between the gal E and gal T genes.

(c) The bacteriophage λ genome codes for a regulatory function (N) which controls transcription of phage RNA presumably by antagonizing the host transcription termination factor rho. This function will allow transcription to elongate beyond termination signals. Study of this phage promoted transcription should help in understanding the process of termination of transcription. A phage transcript was isolated and found to be (1) very large, and (2) unusually stable. Current studies aim at (1) understanding the basis for this abnormal stability, and (2) determining the precise role of N in antitermination.

Significance to Biomedical Research and the Program of the Institute: Studies on nucleotides sequences of the regulatory segments of DNA are just starting in several laboratories. Our aim is to obtain the nucleotide sequence of all the regulatory regions of the gal operon. These regions include the sites for binding of RNA polymerase, for initiation of transcription, for repressor binding, for binding and action of termination factor(s). They will also include the ribosomal binding sites on gal mRNA. Information stemming from our studies as well as analogous studies on other bacterial operons will indicate the genetic codewords for regulation of transcription. Precise understanding of gene expression in microorganisms should provide a basis for understanding how genes are controlled in normal and transformed cells.

Proposed Course: 1. Determine the nucleotide sequences of the gal operon, both the initiation and termination regions of transcription. Study the precise interaction between the regulatory proteins and their binding sites on gal DNA. 2. Search for and purify the regulatory molecules which control termination of transcription in E. coli and determine their mode of action. Study mutants affected in these functions. 3. Search for molecules active in the coupling of translation to transcription in E. coli.

Honors and Awards: None

Publications:

de Crombrugghe, B. and Pastan, I.: Regulation of Gene Expression by Cyclic AMP in Escherichia Coli. In Davies, D.D. (ed.): Rate Control of Biological Processes. Cambridge, England, Cambridge University Press, 1973, pp. 365-369.

Adhya, S., Gottesman, M. and de Crombrugghe, B.: Termination and Antitermination in Transcription: Control of Gene Expression. In Proceedings of the "Symposium on Control of Transcription", Bose Institute, Calcutta, India, in press, 1973.

Adhya, S., Gottesman, M. and de Crombrugghe, B.: Release of Polarity in E. coli by Gene N of Phage Lambda: Termination and Antitermination of Transcription. Proc. Nat. Acad. Sci., U.S.A., in press, 1973.

Serial No. NCI-8009
1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Factors that Regulate Cyclic AMP Levels in Cultured Cells

Previous Serial Number: None

Principal Investigator: George S. Johnson

Other Investigator: None

Cooperating Units: None

Man Years: July 1, 1973 through June 30, 1974

Total:	1.0
Professional:	1.0
Others:	0

Project Description:

Objectives: To determine which of the agents known to affect growth and morphology affect cyclic AMP levels and thus possibly exert their effects through cyclic AMP, and to determine if transformed cells are less susceptible to control by these agents.

Methods Employed: Standard cell culture and radioimmunoassay techniques will be used.

Major Findings: Transformed cells grown in culture have low levels of cyclic AMP and many abnormal properties of these cells can be attributed to these low levels. Following addition of dibutyryl cyclic AMP to the growth medium of transformed cells many abnormal properties return towards normal. These changes are induced by analogs of cyclic AMP but not other structurally similar chemicals, and also by agents which elevate cyclic AMP levels. Thus the effects are specific for cyclic AMP. However, we have found one major exception to this rule. The derivatives of adenine substituted at the N-6 position rapidly induce cell elongation without an elevation of the cyclic AMP levels. At present, the mechanism of cell elongation induced by the N^6-substituted adenines is not clear but is under study. Not all abnormal properties of transformed cells are altered by elevation of the cyclic AMP levels. The NAD$^+$/NADH ratio is one such property. The NAD$^+$/NADH ratio increases as normal cells divide to high densities. However, in transformed cells, the NAD$^+$/NADH ratio is low and independent of cell density. Elevation of the intracellular cyclic AMP level does not alter the ratio in either normal or transformed cells.

Significance to Biomedical Research and the Program of the Institute: By understanding better how growth is regulated in normal cells and how this regulation is altered by transformation, we may learn how to control the growth of certain cancer cells.

Proposed Course: We will study the ability of growth promoting agents to initiate growth in quiescent cells and to stimulate growth in growing cells. We will then measure the cyclic AMP level in the stimulated and unstimulated cells.

Honors and Awards: None

Publications:

Rein, A., Carchman, R., Johnson, G.S., and Pastan, I.: Simian Virus 40 Rapidly Lowers cAMP Levels in Mouse Cells. Biochem. Biophys. Res. Commun. 52: 899-904, 1973.

Johnson, G.: Regulation of Cell Functions in Fibroblasts by Cyclic AMP. In The Role of Cyclic Nucleotides in Carcinogenesis, New York, Academic Press, 1973, Vol. 6, pp. 39-45.

Pastan, I., Anderson, W.B., Carchman, R.A., Willingham, M.C., Russell, T.R. and Johnson, G.S.: Cyclic AMP and Malignant Transformation. In B. Clarkson and R. Baserga (eds.): Control of Proliferation in Animal Cells. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, in press, 1974.

Carchman, R.A., Johnson, G.S., Pastan, I., and Scolnick, E.M.: Studies on the Levels of Cyclic AMP in Cells Transformed by Wild-Type and Temperature-Sensitive Kirsten Sarcoma Virus. Cell 1: 59-64, 1974.

Johnson, G.S., D'Armiento, M., and Carchman, R.A.: N^6 Substituted Adenines Induce Cell Elongation Irrespective of the Intracellular Cyclic AMP Levels. Experimental Cell Res., in press, 1974.

Schwartz, J.P., Johnson, G.S., Pastan, I., and Passonneau, J.V.: The Effect of Growth Conditions NAD$^+$ and NADH Concentrations and the NAD$^+$/NADH Ratio in Normal and Transformed Fibroblasts. J. Biol. Chem., in press, 1974.

Pastan, I. and Johnson, G.S.: Cyclic AMP and the Transformation of Fibroblasts. In Advances in Cancer Research, in press, 1974.

Johnson, G.S. Cyclic AMP and Cellular Transformation. In Proceedings of the Princess Takamatsu Cancer Symposium, Tokyo, Japan, in press, 1973.

SUMMARY REPORT

LABORATORY OF PHYSIOLOGY

JULY 1, 1973, THROUGH JUNE 30, 1974

The research effort of the Laboratory of Physiology can be subdivided into five general compartments: (1) Investigation of the mechanisms of thyroid hormone release and maintenance of iodine levels; (2) Metabolism studies of the regulation and energy expenditures as well as the interdependence of metabolic and behavioral responses; (3) Control of the synthesis of proteins and nucleic acids; (4) Differences in nucleic and metabolism between normal and tumor cells; and (5) Molecular and biological effects of radiation.

It has been established that some mitochondria are intimately associated with desmosomes basal to the junction of thyroid epithelial cell. The anchoring of some mitochondria may be involved in supplying local concentrations of ATP to areas of plasma membrane. In the area of transplantable thyroid tumors, it is now clear that the typical thyroid epithelial cell is derived from the ventral outpocketing of the pharynx. The investigation of the membranes of thyroid epithelial cells showed that all membranes contained intramembrane particles, the number per unit area being higher on the cytoplasmic fracture face than on the complimentary face. The equilibrium concentration of Ca-45 was higher in thethyroid gland than in the blood of hypophysectomized rats. In the area of radioisotope studies it appeared that apical membrane is pumping radioiodide into the cells from the lumen.

The objectives of the metabolism studies covered investigation of how the energy expenditure, feeding and general behavior pattern are related to tumor growth, studies of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth, and development of quantitative and reproducible assessment of behavioral output, with hope of detecting early systemic effects of tumors, as well as searching for experimental models for cachexia and decline in food intake observed during neoplastic growth.

Part of the cachectic decline of food intake in some cancers is attributable to decline in effective energy flow of the host with resultant decline in energy available for motor activity. The response of components of water and energy exchange and of food intake to levels of salt loading showed that salt depletion of the host by the tumor is not a substantial cause of the fall in food intake or motor activity. Work in progress on feeding response to reduced environmental temperature indicates that this response, as well as that to nutrient density, is progressively attenuated with tumor growth. The persistent feeding deficits in animals recovered from lateral hypothalamic damage can be clearly discriminated from cachectic feeding failure. The extent to which food intake is modified by change in amount of feeding activity and by change in feeding efficiency is being examined. The tumor-bearer opposes the cachectic process by use of an active behavioral radicals. However, the radiation-induced destruction of sulfur radicals is decreased substantially by the presence of nickel ions.

Quantitative studies of recovery from sublethal irradiation in terms of ability to tolerate a second challenging irradiation show separate patterns for hematopoietic and gastrointestinal systems. Ability to tolerate other agents will also vary according to time after irradiation and system affected. An understanding of the nature and duration of effects of each treatment or combination of treatments is essential in establishing therapeutic programs which can be tolerated by the host.

Since the replication of DNA is an obligatory prerequisite for cell division, the study of DNA synthesis and its control occupies a position of unique importance in cancer research because the malignant cancer cell displays continuous and uncontrolled division. An attempt was made to isolate and identify deoxyribose-containing compounds other than DNA from normal, precancerous, and tumor tissues, to determine the metabolic sequences in which these compounds are involved in normal, precancerous and cancer tissues; to isolate DNA from subcellular structures other than nuclei, and to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

Finally, investigation of nucleotide metabolism in normal and cancer cells was carried out. In mammalian tissues uridine-cytidine kinase is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction is rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end products. This enzyme was purified 150-fold from the mouse ascites mast cell neoplasm P815/S and characterized with respect to stability, pH optimum, divalent cation requirement, and substrate specificity. The enzyme exhibited a broad specificity for the phosphate donor substrate, but a lesser one for the phosphate acceptor substrate. The ratio of uridine to cytidine kinase activity remained constant throughout the purification of the enzyme. The purified enzyme has a molecular weight of at least 150,000. Kinetic studies are now in progress.

The UMP-CMP kinase has been purified 300-fold from *Tetrahymena pyriformis*, a rapidly growing protozoan unable to synthesize the pyrimidine ring *de novo* and hence dependent on anabolism from the preformed ring. The purified *Tetrahymena* enzyme also retains capacity to phosphorylate dCMP. This is the first extensive purification for CMP-UMP kinase activity; this enzyme has now been renumbered as EC 2.7.4.14, ATP: (d)CMP phosphotransferase. This purified enzyme was most stable in phosphate buffer, had a pH optimum between 7.0 and 8.0, required Mg^{2+}, was inhibited by CDP, UDP, ADP, and high levels of ATP·Mg. Subsequent studies revealed that the mechanism of the enzymatic reaction was probably sequential by addition of both substrates to the enzyme to form a ternary complex. Although the reaction is freely reversible, physiologically the enzyme is intended to function primarily in the forward or anabolic direction. The sequential addition of CMP and $ATP \cdot Mg^{2-}$ to the enzyme occurs in a random fashion.

In the course of the year, major synthetic efforts were successfully accomplished. Stockpiles of the four isomers of β-hydroxynorleucine have been increased in anticipation of their use in anti-tumor studies. The cause of low yields in the resolution procedure was investigated. The structure of β-hydroxymethoxinine and of β-hydroxyhomomethionine has been confirmed by infrared absorption studies, nuclear magnetic resonance spectra, and mass spectrometry. Optically pure L-norleucine and D-norleucine and their N-chloroacetyl derivatives have been prepared by enzymatic resolution of chloroacetyl-DL-norleucine. N-Chloroacetyl derivatives of DL-allylglycine and of DL-β-2-thienylalanine were prepared and their hydrolytic rates with Acylase I and with pancreatic carboxy-peptidase A, respectively, were determined as a preliminary step in the preparation of the optically pure amino acids and their N-acyl derivatives. The racemic diastereomeric mixture of β-chloroacetyl-β-hydroxyaspartic acid has been prepared.

Studies continue on immunogenetic factors influencing bone marrow transplantation in mice, with the goal of avoiding GVH disease, so that this procedure can become an adjunct to heavy radiation or chemotherapy in the treatment of cancer. Marrow from the mother produces a less severe reaction in radiated mouse progeny than that from the father, presumably because of the persistence of tolerance for antigenic differences between mother and fetus. It was also noted that radiated daughter mice were more tolerant of mothers' marrow than radiated sons; this was true both for inbred and hybrid strains, suggesting that it is due to antigens controlled by the Y chromosome. These phenomena have now been studied by transplantation of fertilized ova of one strain into foster mothers of another. The results generally confirm the idea that phenotypic expression of the antigens of the fetus, and responsiveness as well, is reduced by maternal influences.

Research continues on the effects of ionizing radiation on nucleic acids, proteins and their constituents. Radioprotective and radiosensitizing agents are being studied in order to develop new approaches to increase the differential radiation sensitivity of tumors. The protective effects of certain metal ions and of their complexes in reducing the production of free radicals in gamma-irradiated DNA have been further investigated. At metal to nucleotide ratios of 1:10, a 3- to 10-fold decrease of the electron spin resonance signal due to carbon radicals was observed, independent of dose and temperature. It appears that the metal ions act by quenching excited states and/or by charge scavenging, and that either charge or energy can migrate over long distances in these solids.

The effect of metal ions on the gamma-radiolysis of polycrystalline djenkolic acid was investigated in detail by electron spin resonance. This substance is of interest because it allows one to separate the effects of metal ions on the formation of carbon radicals, their thermal decay, their conversion to sulfur radicals and the gamma-radiation induced destruction of the sulfur radicals. All of these processes occur in gamma-irradiated proteins, where the effect of metal ions on these four different processes is difficult to disentangle. For djenkolic acid it was found that certain metal ions decrease the carbon radical formation, increase the second order rate constant for radical recombination during thermal radical decay, but in general do not affect the rate constant for the conversion of carbon to sulfur regulatory variant so far not found in noncancerous states.

In the area of protein and macromolecular synthesis, the objectives were to study the controls exercised by normal and tumor cells in regulating synthetic processes and to investigate metabolic transformations of nucleic acids. When certain amino acid analogs are incorporated into globin, the resulting protein is rendered unstable and susceptible to proteolytic degradation. Various analogs with hetero atoms, Cl, S, N, or O attached to the β-carbon atom are particularly effective in this regard. Such amino acids, when incorporated in place of their natural counterparts, promote the breakdown of helical portions of the newly formed protein. Thus denatured, the proteins become substrates for a variety of proteolytic enzymes. It has been established that hemin control of globin synthesis becomes retarded by translational repressor(s) which inhibit peptide chain initiation. These repressors are formed in the riosome-free supernatant fraction from available precursor, pro-repressor. Hemin blocks this conversion, but only in the early stages of its formation can the repressor be inactivated by hemin. Both pro-repressor and repressor are proteins with estimated mol. wts. of 3×10^5 daltons. The conversion of pro-repressor to repressor is markedly temperature dependent. The repressor inhibits initiation of α- and β-chains of globin as well as that of minority proteins. Derived protein synthetic systems and partial systems lose the receptor required for repressor function and therefore are no longer under hemin.

As a result of previous work on methionineless death in E. coli, it was of interest to demonstrate restriction activity in vitro by one measuring the change in sedimentation rate of small circular DNA's as they are cleaned. Two E. coli strains were employed: Strain $15T^-$(555-7) and strain 2366, a mutant of $15T^-$(555-7). From each strain a relatively crude restriction enzyme was prepared by ammonium sulfate fractionation. Incubation of each of the restriction enzyme preparations with a mixture of radioactive plasmids indicated that nuclease activity was present only in the extract from strain 557-5 and only against plasmids from 2366, showing that strain 2366 is devoid of the "15" host modification activity. Under conditions of thymine starvation of a thymine auxotroph of E. coli there is a 10% increase in net DNA. This increase occurs over a period of one hour and is followed by degradation of DNA. The DNA synthesized during thymine starvation contains large amounts of dUMP instead of dTMP. It would appear that it is this incorporation that causes the subsequent DNA degradation and death of the cell.

Earlier experiments have shown that some of the β-hydroxy-amino acids exhibited growth-inhibitory activity in microbial systems selected for antitumor screening, and probable tumor-inhibitory action in some mammalian systems. Attempts were made to prepare β-hydroxynorleucine, to isolate its four isomers, and to prepare the N-chloroacetyl derivatives of these isomers. Further, other related β-hydroxyamino acids, such as the methionine derivatives, β-hydroxymethionine, β-hydroxyhomomethionine, β-hydroxymethoxinine, chain-substituted halo-aliphatic amino acids, such as gamma-dichlorothreonine, gamma-trifluorothreonine, and gamma-chloro-β-hydroxyleucine, were to be synthesized in order to test these compounds for anti-tumor activity, first in microbial screening system, and then in mammalian system.

Serial No. NCI-905
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Iodide-Concentrating Mechanism of the Thyroid Gland

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. Lois W. Tice, Laboratory of Experimental Pathology, National Institute of Arthritis and Metabolic Diseases, NIH, Bethesda, Md. (Serial No. NIAMD, LEP-10)

Dr. André Haeberli, Laboratory of Physiology, National Cancer Institute, NIH, Bethesda, Md.

Man Years:

Total:	2.0
Professional:	1.0
Others:	1.0

Project Description:

Objectives: To learn about (a) the mechanism by which the thyroid gland maintains a concentration of iodide elevated above that in the blood, and (b) the mechanism by which the thyroid gland accumulates protein-bound iodide.

Major Findings: Structure of plasma membrane: The plasma membrane and other membranes of the typical thyroid epithelial cell of the rat were examined using the freeze-fracture technique. Intramembrane particles were found on all membranes although the number per unit area was much higher on the cytoplasmic fracture face of the plasma membrane than on the complementary face. Tight junctions which act as seals between cells (preventing the thyroglobulin from leaking out of the lumen) were observed. An unusual feature found was that the structure of the tight junction depended upon the activity of the gland. Gap junctions (sites of conductivity between cells) were observed on the lateral plasma membrane, but not associated with tight junctions.

Calcium: Equilibration of calcium-45 between thyroid gland and blood was studied. The equilibrium concentration in the thyroid was several times that in the blood of the hypophysectomized rat, and was progressively less the more active the gland. Rates of equilibration were greater the more active the gland, and much less than for radioiodide.

Radioiodide transport: Comparison was made between the electrical potential difference between the follicular lumen and cells and the corresponding equilibrium radioiodide concentration ratios. In general, the concentration ratios were radically different from those expected from the Nernst equation and indicated that the apical membrane is pumping radioiodide into the cell from the lumen.

Significance for Cancer Research and the Program of the Institute: Some of the work reported here may best be put into Objective 3, Approach 5 because they give information on properties of cell surface and cell membranes.

Publication:

Tice, L., and Wollman, S. H.: Ultrastructural localization of peroxidase on pseudopods and other cell structures of the typical thyroid epithelial. Endocrinology (in press).

Serial No. NCI-906
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Production and Properties of Transplantable Thyroid Tumors

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. S. R. Hilfer, Department of Biology, Temple University, Philadelphia, Pennsylvania

Man Years:

Total:	0.5
Professional:	0.5
Others:	0

Project Description:

Objectives: To study thyroid growth and the production and properties of transplantable thyroid tumors in the rat: changes occurring in the thyroid during growth, hyperplasia and involution; histologic types of thyroid tumors and their progression, and the correlation of histology with the biochemistry and physiology of the tumors. We are also studying a variety of cells recently recognized to be present in the normal thyroid gland and which may be the cells of origin of some of the different histologic types of thyroid tumors observed in the past.

Major Findings: At least 3 different types of epithelial cells are found in the thyroid gland of the rat and five in the thyroid of the mouse. The thyroid has a multiple origin being derived from the fusion of a ventral outpocketing and two lateral outpocketings (ultimobranchial bodies) from the pharynx. To see which cells are derived from the ventral outpocketing and which from the ultimobranchial body these rudiments have been transplanted separately, before their fusion, into the kidney capsule of the adult. It is now clear that the typical thyroid epithelial cell is derived from the ventral contribution. Investigation of the sources of the other cells is continuing.

Significance for Cancer Research and the Program of the Institute: The properties of cells and of the tumors derived from them frequently depend upon the embryologic origin of the cells. This project will allow us to understand better the properties of certain tumors.

Publication:

Nève, P. and Wollman, S. H.: Crystals in dense bodies in the typical thyroid epithelial cell of the I mouse. J. Natl. Cancer Inst. 51: 659-665, 1973.

Serial No. NCI-928
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intracellular Colloid Droplets

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. Lawrence H. Bernstein, Research Associate

Man Years:

Total:	1.5
Professional:	1.5
Others:	0.

Project Description:

Objectives: To determine the mechanism of thyroid hormone release.

Major Findings: We have found that some mitochondria are intimately associated with some desmosomes (modifications of the plasma membrane) especially some of the desmosomes just basal to the tight junction of the typical thyroid epithelial cell. The mitochondria appear to be held to the desmosomes by fibrils. The association appears to occur in both active and inactive glands. The anchoring of some mitochondria might serve the purpose of supplying a local high concentration of ATP to some areas of the plasma membrane of the cell.

Significance for Cancer Research and the Program of the Institute: This project fits most closely under Objective 3, Approach 5, having to do with properties of the cell surface.

Publication:

Wollman, S. H. and Loewenstein, J. E.: Rates of colloid droplet and apical vesicle production and membrane turnover during thyroglobulin secretion and resorption. Endocrinology 93: 248-252, 1973.

Serial No. NCI-925
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Protein Synthesis and Its Control in Normal and Tumor Cells

Previous Serial Number: Same

Principal Investigator: Marco Rabinovitz

Other Investigators: Dr. Saburo Ayukawa, Visiting Fellow

Man Years:

Total:	5.5
Professional:	2.0
Others:	3.5

Project Description:

Objectives:

(a) To study, in both normal and tumor cells the controls exercised by these cells in regulating the synthesis of protein. This is generally made through an analysis of the biochemical lesions introduced within the pathway by deficiencies of essential nutrients or by inhibitors.

(b) Where applicable, to extend the information obtained in the first objective to the tumor-bearing animal under controlled nutritional conditions, with the view of specific inhibition of protein synthesis by cancer cells or to other selective lethal effects.

Major Findings:

Hemin Control of Globin Synthesis: As a result of hemin deficiency, globin synthesis in rabbit reticulocytes becomes retarded by translational repressor(s) which inhibit peptide chain initiation. These repressors are formed in the ribosome-free supernatant fraction from available precursor, pro-repressor. Hemin blocks this conversion, but only in the early stages of its formation can the repressor be inactivated by hemin. During the linear production of reversible repressor there is a lag in the formation of irreversible repressor, suggesting the precursor product relationship:

$$\text{pro-repressor} - \text{hemin complex} \rightleftarrows \text{pro-repressor} \rightleftarrows \begin{array}{c}\text{reversible}\\ \text{repressor}\end{array} \rightarrow \begin{array}{c}\text{irreversible}\\ \text{repressor}\end{array}$$

$$\begin{array}{c} + \\ \text{hemin} \\ + \\ \text{globin} \rightarrow \text{methemoglobin} \end{array}$$

Both pro-repressor and repressor are proteins with estimated mol. wts. of 3×10^5 daltons. The conversion of pro-repressor to repressor is markedly temperature dependent and occurs over a period of several hours at 34°C without the requirement of compounds of low molecular weight. The reaction is promoted by sulfhydryl reagents, which soon thereafter cause inactivation of the repressor. The data suggest that conversion of pro-repressor to repressor may be a conformational change which is retarded by hemin.

The repressor inhibits initiation of α- and β-chains of globin as well as that of minority proteins. Derived protein synthetic systems and partial systems lose the receptor required for repressor function and therefore are no longer under hemin control. The repressor acts by preventing the initiation codon dependent interaction of the 40S and 60S ribosomal subunits. The block appears to be associated with an initiation codon dependent deacylation of ribosomal bound met-$tRNA_f$.

Effect of Amino Acid Analogs on Structure of Newly Formed Globin: When certain amino acid analogs are incorporated into globin, the resulting protein is rendered unstable and susceptible to proteolytic degradation. Various analogs with hetero atoms, Cl, S, N, or O attached to the β-carbon atom are particularly effective in this regard. These data are in conformity with reports in the literature indicating that such amino acids do not support the formation of a polypeptide α-helix, and suggest that when incorporated in place of their natural counterpart, they promote the breakdown of helical portions of the newly formed protein. Thus denatured, the proteins become substrates for a variety of proteolytic enzymes.

Significance for Cancer Research and the Program of the Institute: These studies are designed to help explain the nature of the lesions caused by nutritional deficiencies and antagonisms at the level of macromolecular biosynthesis. Such evaluations should provide a basis for our understanding of control of protein-forming systems in various cell types. This project thus conforms to the NCI research plan as follows: Objective 3, Approach 1, nature and modification of precancerous state to determine mechanisms accounting for high degrees of stability of cell functioning. Also, the study of mechanism of action of analogs of amino acids on protein structure and stability furnishes a theoretical basis for Objective 6, Approach 2, discover new therapeutic agents and develop means for their selection.

Publications:

Baikow, K., Mizuno, S., and Rabinovitz, M.: Inhibition of an initiation codon function by hemin deficiency and the hemin-controlled translational repressor in the reticulocyte cell-free system. Biochem. Biophys. Res. Commun. 54: 315-323, 1973.

Gross, M., and Rabinovitz, M.: Control of globin synthesis by hemin: Effect of temperature on globin synthesis by the reticulocyte cell-free system and the activity of translational repressors formed in the absence of hemin. Biochim. Biophys. Acta 299: 472-479, 1973.

Baikow, K., and Rabinovitz, M.: Increased binding of transfer ribonucleic acid species to ribosomes under conditions interfering with their aminoacylation. Mol. Pharm. 9: 229-236, 1973.

Baikow, K., Mizuno, S., Fisher, J. M., and Rabinovitz, M.: Hemin control of globin synthesis: Effect of a translational repressor on MET-tRNA$_f$ binding to the small ribosomal subunit and its relation to the activity and availability of an initiation factor. Biochim. Biophys. Acta 324: 397-409, 1973.

Rabinovitz, M.: Translational repression in the control of globin chain initiation by hemin. Ann. N. Y. Acad. Sci. (in press).

Serial No. NCI-948
1. Laboratory of Physiology
2. Physical Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Control of Macromolecular Synthesis

Previous Serial Number: Same

Principal Investigator: Theodore R. Breitman

Man Years:

Total: 3
Professional: 1
Others: 2

Project Description:

Objectives: To clarify metabolic transformation of nucleic acids and their nucleotide precursors.

Major Findings:

A. DNA modification and restriction

As a result of our previous work on methionineless death in *E. coli*, it was of interest to demonstrate restriction activity *in vitro*. Because of the very limited action of restriction enzymes (they make a double-strand break in the DNA) very sensitive assays are needed to detect activity. One assay is to measure the change in sedimentation rate of small circular DNA's as they are cleaned.

For this project two *E. coli* strains were employed. Strain $15T^-$(555-7) which was reported to contain at least two independent host DNA modification systems: "A," located on the bacterial chromosome, and "15," located on a plasmid. The other strain was 2366, a mutant of $15T^-$(555-7) which from genetic evidence, has lost the "15" host modification system. The DNA of each strain was labeled with radioactive thymidine [^3H for 2366 and ^{14}C for $15T^-$(555-7)] and the plasmids isolated by equilibrium centrifugation in a CsCl-ethidium bromide gradient.

From each strain a relatively crude restriction enzyme was prepared by ammonium sulfate fractionation.

Incubation of each of the restriction enzyme preparations with a mixture of radioactive plasmids followed by centrifugation through a sucrose density gradient indicated that nuclease activity was present only in the extract from strain 557-5 and only against plasmids from 2366. These results provided direct evidence that strain 2366 is devoid of the "15" host modification activity.

B. Composition of the DNA synthesized during thymine starvation

It has been known for some time that _in vitro_ the DNA polymerases can incorporate dUMP into DNA instead of dTMP. We have now obtained direct evidence that this replacement also occurs _in vivo_.

Under conditions of thymine starvation of a thymine auxotroph of _E. coli_ there is a 10% increase in net DNA. This increase occurs over a period of one hour and is followed by degradation of DNA.

We have now found that the DNA synthesized during thymine starvation contains large amounts of dUMP instead of dTMP. From circumstantial evidence, it would appear that it is this incorporation that causes the subsequent DNA degradation and death of the cell.

Significance for Cancer Research and the Program of the Institute: Findings pertaining to an elucidation of the mechanisms involved in the modification of nucleic acids are important as they may suggest possible means of therapeutic control of cellular growth. Objective 3, Approach 3.

Proposed Course: The principal investigator will be absent during most of the next fiscal year. Continuance of these projects will be temporarily halted during this interim.

Serial No. NCI-380
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Deoxyribose containing compounds in normal, regenerating and cancer tissues

Previous Serial Number: Same

Principal Investigator: Walter C. Schneider

Man Years:

Total:	3
Professional:	1
Others:	2

Project Description:

Objectives: Since the replication of DNA is an obligatory prerequisite for cell division, the study of DNA synthesis and its control occupies a position of unique importance in cancer research because the malignant cancer cell displays continuous and uncontrolled division. The objectives of this project are to isolate and identify deoxyribose-containing compounds other than DNA from normal, precancerous, and tumor tissues, to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal precancerous and cancer tissues; to isolate DNA from subcellular structures other than nuclei, and to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

These studies are aimed at uncovering differences between normal and malignant cells that can be exploited to prevent the formation of cancer cells or to promote the destruction of cancer cells already generated. (These studies support most closely Objective 3: To prevent conversion of cells to those capable of forming cancer and Approach 1: Nature and modification of the precancerous state, of the National Cancer Plan.)

Methods: Nuclei, mitochondria, zymogen granules, and microsomes were isolated from rat or mouse tissues by differential and/or density gradient centrifugation as well as by centrifugation in the zonal rotor. Nucleic acids were extracted from tissue fractions using sodium lauryl sulfate and phenol. DNA was purified from these extracts by treatment with ribonuclease and passage through Biogel P-60, by banding in CsCl, or by treatment with NaOH to remove RNA and small molecules.

DNA samples were examined by means of analytical CsCl density gradient centrifugation, melting behavior, and amount of labeled thymidine incorporated. DNA was measured by a modification of the diphenylamine reaction. Enzyme markers were used for estimating purity of isolated subcellular fractions.

Major Findings: Due to the fact that it was necessary to move the laboratory from Bldg. 37 to Bldg. 10, the amount of research work that was performed during the year was markedly reduced, since a considerable amount of time was needed for planning the new laboratories as well as for the actual move. Unfortunately, the plans that were made for Bldg. 10 could not be implemented prior to the move. This means that research must be conducted under less than adequate conditions for some time and that a further disruption in work must occur when the renovations are made.

During the year work has continued on mitochondrial and microsomal DNA. The detergent method described last year for the separation of these two types of DNA from liver without isolating mitochondria and microsomes has been improved and experiments are in progress to differentiate the two types of DNA by methods other than incorporation of thymidine. These include sedimentation analysis, buoyant density determination, and reassociation rate.

Since reports have appeared in the literature recently demonstrating that non-mitochondrial cytoplasmic DNA of tissue cultures was produced in the nucleus, attempts were again made to see whether a microsomal like DNA could be extracted from nuclei. These experiments gave ambiguous results, since in some experiments DNA similar to microsomal DNA could be extracted while in others it could not. A problem in all these experiments is the fact that isolated nuclei lose DNA as a result of almost any manipulation. Therefore if a microsomal DNA is lost from nuclei it could readily be masked by the loss of other types of nuclear DNA as well.

Significance for Cancer Research and the Program of the Institute: These experiments most closely support Objective 3, Approach 1 of the National Cancer Plan since they deal with the nature of cytoplasmic DNA in precancerous liver. Since little or no work has been done on the effect of the carcinogenic process on mitochondrial and microsomal DNA, the role that they play in this process is not known. Furthermore, since the biological function of these two types of DNA also remains to be established, critical evaluation of these nucleic acids must await further work.

Proposed Course of Research: During the coming year it is hoped to complete a number of unfinished projects. These include the detergent method for separating cytoplasmic DNA, the reassociation studies of mitochondrial and microsomal DNA, the isolation of zymogen granules in the zonal centrifuge, studies of DNA in melanin granules, and studies of cytoplasmic DNA in superovulated ovaries. Studies on cytoplasmic DNA in mammary epithelial cells undergoing hormone stimulation are also in progress.

Serial No. NCI-5221
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies comparing the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with comparable tissues that become neoplastic.

Previous Serial Number: Same

Principal Investigator: Jean Rotherham

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To discover if there are differences in the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with respect to comparable tissues that are becoming neoplastic.

Methods Employed: Suitable chemical agents will be used which enhance the occurrence of neoplastic changes in mouse and/or rat tissues. Nuclear fractions will be isolated and examined for possible differences in metabolic modifications of the nucleoproteins of tissues of treated and control animals.

Major Findings: Since the initiation of this project one year ago, progress has been made in modifying methods suitable for obtaining nuclear preparations from mouse epidermis as well as methods for the isolation and purification of the required material from these preparations, and for the fractionation of the purified protein. For one series of experiments purified material from both control and treated animals has been obtained and this material is ready for fractionation.

Significance for Cancer Research: (See objectives). National Cancer Program Plan Objective and Approach: Cause and prevention; nature and rate of oncogenic cell transformations in carcinogenesis (3, 2).

Proposed Course: Some of the methods to be used for analysis of the purified protein fractions will require examination following which analyses of the material thus far obtained will be made.

Serial No. NCI-5205
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on biochemical mechanisms of sensitivity and resistance to antimetabolites and mechanisms for control of nucleic acid synthesis

Previous Serial Number: Same

Principal Investigator: Elizabeth P. Anderson

Other Investigators: Alec S. Liacouras

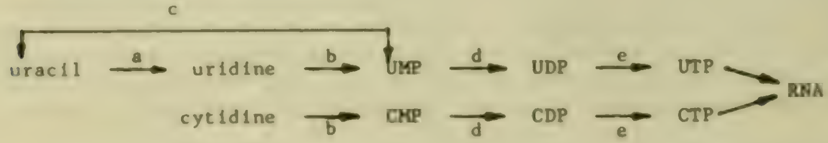
Cooperating Units: F. K. Millar, Laboratory of Theoretical Biology

Man Years:

Total: 2 + 1/2 (STRIDE)
Professional: 1
Others: 1 + 1/2 (STRIDE)

Project Description:

Objectives: The objective of this project is to investigate nucleotide metabolism in normal and cancer cells. The aim is to elucidate pathways of biosynthesis, mechanisms of control over these pathways, and biochemical mechanisms of sensitivity and resistance to purine and pyrimidine analogs. The end-products of nucleotide biosynthesis are the triphosphate precursors of nucleic acids, and regulation of the enzymes involved in these biosynthetic steps is probably a major factor in determining the rate of cellular nucleic acid synthesis. In view of the central role of UMP in pyrimidine metabolism, work has been focussed on reactions b and d in the following pathway of pyrimidine nucleotide biosynthesis:



Methods Employed and Major Findings:

1) Studies on uridine-cytidine kinase. In mammalian tissues uridine-cytidine kinase (reaction b above) is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction has been shown to be rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end-products (Anderson *et al*., Biochim. Biophys. Acta 91: 380, 1964). We have now purified this enzyme 150-fold from the mouse ascites mast cell neoplasm P815/S, in which we first demonstrated feedback regulation of the enzyme. We have characterized the purified enzyme with respect to stability, pH optimum, divalent cation requirement, and substrate specificity. The enzyme exhibited a broad specificity for the phosphate donor substrate; ATP and dATP were the most active, but all nucleoside triphosphates tested, including dUTP and dCTP, were able to serve as donors. This is in striking contrast to the feedback inhibition of the enzyme by the pyrimidine ribonucleoside triphosphate end-products CTP and UTP. Sensitivity of the enzyme to this feedback inhibition was fully retained during the purification. Specificity of the purified enzyme for the phosphate acceptor substrate was less broad; in addition to the substrates uridine and cytidine, only 5-fluorouridine and the 5-aza- and 6-aza-analogs of uridine and cytidine were phosphorylated by the enzyme; deoxyribonucleosides were not phosphorylated, nor was cytosine arabinoside. The ratio of uridine to cytidine kinase activity remained constant throughout the purification of the enzyme. The purified enzyme has a molecular weight of at least 150,000. Initial velocity studies were carried out with this purified preparation, using a spectrophotometric assay that followed the reaction continuously. With either uridine or cytidine as phosphate acceptor, and ATP as donor, the initial velocity pattern was one of intersecting lines, indicating that the reaction proceeds by the sequential addition of both substrates to the enzyme to form a ternary complex, followed by the sequential release of the two products. Analysis of the data by Cleland's Sequen computer program indicated a K_m of the enzyme for uridine of 1.5×10^{-4} M, for cytidine of 4.5×10^{-5} M, and for ATP, with either uridine or cytidine as phosphate acceptor, of 3.6×10^{-3} M or 2.1×10^{-3} M, respectively. The V_{max} was 1.83 μmoles phosphorylated/min/mg enzyme protein for the uridine kinase reaction and 0.91 μmole for the cytidine kinase reaction. Thus, although the rate of uridine phosphorylation was twice that for cytidine, the K_m of the enzyme for cytidine is only one-third that for uridine. It is also of interest that although the activity for uridine was twice that for cytidine when the enzyme was assayed at 37° with different buffers and pH's, a lower temperature altered this ratio of the two activities in favor of uridine. Kinetic studies are currently in progress to explore the nature of the competition between these two alternate substrates. The results should indicate whether both nucleosides are phosphorylated at the same catalytic site on the enzyme or whether the two phosphorylations are mediated by separate sites or isozymes. These experiments should also define whether the greater V_{max} or the lower K_m is the more definitive factor in a nucleoside being a "better" substrate for the enzyme. The low affinity of the enzyme for ATP

is also of interest, since this enzymatic activity is high primarily in tumor tissues, which have a high aerobic metabolism and ATP concentration; the enzyme may require such conditions for high levels of activity. Further studies are also now in progress to elucidate details of the feedback regulation of the enzyme. These have demonstrated that inhibition by the end-product CTP is competitive with the phosphate donor ATP. Present experiments are aimed at desensitizing the enzyme to feedback inhibition and dissociating the regulation from the catalytic activity by molecular alteration of the enzyme protein. Two manuscripts reporting some of the results of these studies have been submitted to the J. Biol. Chem.; one is on the purification and characterization of the enzyme, and the second reports the mechanism of the enzymatic reaction. A third manuscript is in preparation on the results of the substrate competition experiments; a fourth will cover the studies on feedback regulation. Mr. Alec Liacouras is also writing up the part of these studies that he has carried out as part of his dissertation for the Ph.D. degree in biochemistry at George Washington University.

In P815/FUR, a 5-fluorouridine-resistant subline of this tumor, we have shown that resistance to the analog is associated with a decreased level of uridine-cytidine kinase activity (Anderson, Cancer Res. 23: 1270, 1963; activity in crude extracts is as low as 10-20% of that found in extracts from the parent sensitive line). In order to compare the kinase from the resistant subline with that from the sensitive line, the enzyme has been purified 20-30 fold from the resistant cells, using methods similar to those developed for the purification from the sensitive tumor. The procedures had to be adjusted to the greater lability of the enzyme form from the resistant cells; this enzyme form, for example, requires higher ionic strength for the protection of activity. Although the resistant-line enzyme was also a high-molecular-weight protein, there was some evidence that it could be dissociated into enzyme forms that differed in molecular size; this heterogeneity could be related to the greater lability. The low level of kinase activity in the resistant subline also required a modification of the assay procedure to afford accurate detection of the activity. A comparative study of the kinases from the two tumor lines showed that their kinetic parameters appear to be identical, as does the mechanism of the reaction. Thus, the initial velocity pattern again indicated a sequential addition of substrates to the enzyme to form a ternary complex. The K_m of the resistant-line enzyme for uridine was 1×10^{-4} M and for ATP 3×10^{-3} M. However, the resistant-line enzyme utilized both dGTP and dATP as phosphate donors as efficiently as ATP, and the role of the triphosphate as an activator for the enzymatic reaction may be different for the two enzymes. Feedback inhibition by CTP and UTP was similar for both enzymes. Both the sensitive-line and resistant-line enzymes could use 5-fluorouridine as substrate, apparently to a comparable extent, so an altered substrate specificity does not appear to explain the resistance to the analog nor to account for the lower enzymatic activity in the resistant cells. These studies were carried out largely by Dr. Jane M. Glick during one year of postdoctoral fellowship in this laboratory.

A manuscript on these results is in preparation.

2) Studies on UMP-CMP kinase. The enzyme that catalyzes reaction *d* above has been purified 300-fold from *Tetrahymena pyriformis* (Ruffner and Anderson, J. Biol. Chem. 244: 5994, 1969); this is a rapidly growing protozoan unable to synthesize the pyrimidine ring *de novo* and hence dependent on anabolism from the preformed ring. The purified *Tetrahymena* enzyme also retains capacity to phosphorylate dCMP, and we have recently suggested (Anderson, *The Enzymes*, vol. IX; 49, 1973) that this enzyme may well be the same as, or similar to, the dCMP kinase purified from calf thymus by Sugino *et al*. (J. Biol. Chem. 241: 961, 1966). This is the first extensive purification for CMP-UMP kinase activity, and based on the results of these two separate reports on the substrate specificity of the respective purified preparations, this enzyme has now been renumbered as EC 2.7.4.14, ATP : (d)CMP phosphotransferase. Early characterization of our purified enzyme revealed that it was most stable in phosphate buffer, had a pH optimum between 7.0 and 8.0, required Mg^{2+}, was inhibited by CDP, UDP, ADP, and high levels of ATP·Mg, and produced a non-linear double reciprocal plot of 1/v against 1/UMP concentration. Subsequent studies revealed that the mechanism of the enzymatic reaction was probably sequential and that "substrate inhibition" by high levels of UMP might have contributed to the shape of this plot; the conclusion as to reaction mechanism was based on the data on isotope exchange in the partial reactions (Garvey, Millar, and Anderson, Biochim. Biophys. Acta 302: 38, 1973). More recently, studies with CMP and ATP as substrates have allowed deduction of the reaction mechanism from the initial velocity pattern, using a continuous spectrophotometric assay that removed the ADP product in coupled enzymatic reactions. The pattern observed was one of intersecting lines, indicating that the reaction does indeed proceed by sequential addition of both substrates to the enzyme to form a ternary complex. Analysis of the data by Cleland's Sequen program indicated a K_m of the enzyme for CMP of 7.9×10^{-4} M and for ATP of 2.3×10^{-4} M. The mechanism of the reaction has also been investigated in the reverse direction, starting with CDP and ADP, and using a continuous spectrophotometric assay that measured ATP production in coupled reactions. This initial velocity pattern indicated that the mechanism of the reverse reaction is likewise sequential. Analysis by the Sequen program indicated a K_m of 2.6×10^{-4} M for CDP. The V_{max} was 25.8 μmoles phosphorylated/min/mg enzyme protein for the forward reaction and 5.7 μmoles phosphorylated for the reverse reaction. Thus, although the reaction is freely reversible, the rates suggest that, physiologically, the enzyme is intended to function primarily in the forward or anabolic direction. Product inhibition studies on the forward reaction indicated that inhibition by the product CDP was linear competitive with the substrate CMP and non-competitive with the substrate $ATP \cdot Mg^{2-}$. Our early characterization of the purified enzyme suggested that inhibition by the product $ADP \cdot Mg^{-}$ was competitive with $ATP \cdot Mg^{2-}$. These results indicate that the sequential addition of CMP and $ATP \cdot Mg^{2-}$ to the enzyme occurs in a random fashion; the finding of two competitive product inhibition patterns excludes a required order of substrate addition. Thus, the reaction mechanism has been fully elucidated as random sequential. These studies have been carried out largely by Dr. Thomas Garvey, who continued as a part-time guest worker in the

laboratory until August, 1973. A manuscript is now in preparation reporting the results of the studies with CMP - ATP and with CDP - ADP as substrates for this reaction.

Our STRIDE trainee, Mr. Harry Free, in addition to helping with various research projects in progress, has also been engaged in a research project of his own to follow the levels of UMP-CMP kinase activity at different stages of cell growth. He has measured the kinase activity in developing cultures of Tetrahymena to determine how the enzymatic activity increases during growth. Since UMP is the central compound in pyrimidine nucleotide metabolism, the anabolism of this compound into the triphosphate precursors of nucleic acids should be related to the rapid synthesis of nucleic acids during active cell growth. The study assayed UMP kinase in cell lysates at different stages of culture growth, using a radioactivity assay employed in other experiments in the laboratory. Cell growth was measured both by total protein and by cell count, counting acid-fixed cells in a hemocytometer; this latter aspect was particularly challenging for him and required considerable research for an appropriate method, since live Tetrahymena are transparent to light and highly motile, rendering many usual counting methods useless. The increase in UMP kinase activity closely followed the increase in total protein and in cell count and did not precede the peak of cell growth. After growth leveled off, aging cells did not show an immediate decrease in UMP kinase activity, even though total protein did decrease. The kinase activity must therefore be functional in the maintenance of cell viability, probably in RNA synthesis, and is hence required both for the increased production of RNA and DNA during growth and for the RNA activity throughout cell life. The results of these studies were published in the college's scientific journal.

3) Related activities. In addition to the biochemical studies, the work on both uridine-cytidine kinase and UMP-CMP kinase has made use, for the initial velocity and product inhibition studies, of the DCRT central computer facility for routine calculations and for the more complicated problems of curve fitting, statistical evaluation of the data, and calculation of the K_m's, V_{max}'s, and rate constants for the reactions. In these computer operations, we have had a fruitful collaboration with Dr. F. K. Millar, Laboratory of Theoretical Biology, NCI.

Twice this year we have participated in galactosemia problem-solving sessions for the medical students at the University of Tennessee. This is a new teaching method aimed at re-enacting major research discoveries that communicate the relevance of basic research to medical practice. The process has been so successful in eliciting interest, from the medical students and the medical community, in basic research and in NIH, that this particular problem-solving is now being proposed as a model for current medical school teaching.

Significance to Cancer Research and the Program of the Institute: UMP is the central compound in pyrimidine nucleotide biosynthesis in that it is the precursor for synthesis of other pyrimidine nucleotides of both the ribo- and deoxyribo- series. UMP synthesis by uridine kinase is called into play in rapidly growing tissues such as tumor tissues, and this constitutes a difference between normal and cancer cells. It is important to discover how the enzymes for UMP synthesis and metabolism are regulated in both types of cells, since such regulation is crucial in maintaining a balance among the levels of the various nucleotide precursors of nucleic acids and functions in regulating the rate of cellular nucleic acid synthesis. (NCP Objective 3, Approach 2).

Proposed Course of Research: Further studies on the purified uridine-cytidine kinase from the P815 tumor will aim to answer definitively whether the two normal substrates uridine and cytidine, and the analog substrate 5-fluorouridine, compete for a single, identical catalytic site on the enzyme or whether they are, in fact, phosphorylated by separate sites or isozymes. Additional studies will define the way in which the feedback inhibitions by CTP and UTP compete with ATP and with the respective nucleoside substrates cytidine and uridine. Experiments will also be continued to dissociate the feedback regulation from the catalytic activity by alteration of the enzyme protein molecule. If ways can be found to stabilize the enzyme to further purification, studies on the mechanism of the feedback regulation will also be extended to encompass explorations on the detailed molecular structure of the enzyme.

Mr. Free hopes to extend his study of anabolic enzymatic activities during cell growth to measurements of uridine-cytidine kinase (reaction b) and UMP pyrophosphorylase (reaction c) activities; both of these enzymes precede UMP-CMP kinase (reaction d) in the anabolic pathway in Tetrahymena and either might therefore be rate-limiting in the increase in the pathway during cell growth. With Mr. Free's increased training under the STRIDE program and his increased professionalism, this study also promises to yield fully publishable results.

Publications:

Anderson, E. P.: Nucleoside and nucleotide kinases. In Boyer, P. D. (ed), The Enzymes, 3rd ed., Academic Press, New York, New York, 1973, Vol. IX, 49-96.

Free, H. E.: Exploring the levels of UMP kinase in Tetrahymena pyriformis during different phases of growth. F.C.C. Biologist (Frederick Community College), 1: 94-104, 1973.

Serial No. NCI-306
1. Laboratory of Physiology
2. Nucleic Acids Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of β-hydroxy-α-amino acids

Previous Serial Number: Same

Principal Investigator: Theodore T. Otani

Man Years:

Total:	2
Professional:	1
Others:	1

Project Description:

Objectives: To prepare chemical compounds which preferentially destroy cancer cells by mechanisms other than those of agents presently being used in cancer chemotherapy. Earlier experiments have shown that some of the β-hydroxyamino acids prepared in this Laboratory exhibited growth-inhibitory activity in microbial systems selected for anti-tumor screening, and probable tumor-inhibitory action in some mammalian systems.

More specifically, the immediate objectives of this portion of the project have been:

(a) To prepare β-hydroxynorleucine, which have been shown previously to have possible anti-tumor activity in microbial anti-tumor screening system, to isolate its four isomers, and to prepare the N-chloroacetyl derivatives of these isomers.

(b) To prepare other related β-hydroxyamino acids, such as the methionine derivatives, β-hydroxymethionine, β-hydroxyhomomethionine, β-hydroxymethoxinine; chain-substituted halo-aliphatic amino acids, such as; γ-dichlorothreonine, γ-trifluorothreonine, and γ-chloro-β-hydroxyleucine.

(c) To isolate the four isomers of each of these compounds and to prepare the N-acyl derivatives of each isomer.

(d) To prepare the N-acyl derivatives of other amino acid analogs which have been shown to possess antimetabolic properties.

(e) To test these compounds, both the free amino acids and the N-acyl derivatives, for anti-tumor activity, first in microbial screening system, and then, if found active, and when obtained in sufficient quantities, in mammalian system.

Methods Employed: The β-hydroxyamino acids are prepared by methods developed in this Laboratory. An appropriate aldehyde is condensed with glycine in the presence of catalytic amounts of cupric ions (or with glycine-copper complex) in basic medium under conditions which are empirically determined for each compound.

Major Findings:

(1) Stock-piles of the four isomers of β-hydroxynorleucine are still being increased in anticipation of their use in anti-tumor studies using mammalian systems. The cause of low yields in the resolution procedure is being investigated. It appears that these compounds are absorbed onto charcoal with greater facility than their homologs, threonine and serine, and hence the absorbing agent is either omitted, or used in much smaller quantities than previously.

(2) The structure of β-hydroxymethoxinine, and of β-hydroxyhomomethionine has been further confirmed by infrared absorption studies, nuclear magnetic resonance spectra, and mass spectrometry.

(3) As the N-chloroacetyl derivative of β-hydroxyhomomethionine diastereomer A has been found to be inert to the action of Acylase I, the preparation of a N-trifluoroacetyl-β-hydroxyhomomethionine has been attempted. However, the product could not as yet be obtained in crystalline state.

(4) Optically pure L-norleucine and D-norleucine and their N-chloroacetyl derivatives have been prepared by enzymatic resolution of chloroacetyl-DL-norleucine, since these compounds as obtained commercially were found to be impure.

(5) N-Chloroacetyl derivatives of DL-allylglycine and of DL-β-2-thienylalanine were prepared and their hydrolytic rates with Acylase I and with pancreatic carboxypeptidase A, respectively, were determined as a preliminary step in the preparation of the optically pure amino acids and their N-acyl derivatives.

(6) Several attempts were made to prepare the N-acyl derivative of L-mimosine, a tyrosine analog, without success.

(7) The racemic diastereomeric mixture of β-chloroacetyl-β-hydroxyaspartic acid has been prepared.

Significance for Cancer Research: That amino acid metabolism may be a susceptible area of action in cancer therapy was demonstrated by the efficacy of asparaginase in certain tumors which required asparagine. Amino acid deprivation, either by the removal of the amino acid or by the substitution with an analog may be effective in inhibiting tumor growth.

The unnatural amino acids synthesized under this program are close structural analogs of essential natural amino acids, differing in some cases only in the presence of a single hydroxyl group adjacent to the α-amino group. Hence, β-hydroxyleucine differs from leucine only in the presence of a hydroxyl group in the β-position of carbon chain of the amino acid. β-Hydroxynorleucine is a structural analog of methionine, differing from the latter in having a methylene group in place of the sulfur atom in the γ-position of the chain. Indeed, β-hydroxynorleucine, even as a mixture of four isomers, not only exhibited inhibitory action in two microbial systems, but also showed some anti-tumor effect against Carcinoma 755 as measured by the decrease of tumor size and increase in survival time. β-Hydroxymethoxinine and β-hydroxymethionine are even closer analogs of methionine than is β-hydroxyhomomethionine, the former differing from methionine in possessing an oxygen atom in place of the S atom and in possessing a β-hydroxyl group.

The chain-halogenated amino acids, similarly, are close structural analogs of essential amino acids. For example, γ-dichlorothreonine would be an analog of leucine, since the size of the chlorine atom is approximately that of the methyl group; also γ-triflurothreonine would be a close analog of threonine since the atomic radius of the fluorine atom is essentially that of hydrogen atoms. Objective 6, Approach 2.

Proposed Course of Research:

(1) To increase further the stock-pile of each of the four isomers of β-hydroxynorleucine, and their N-chloracetyl derivatives, for use in anti-tumor studies in mammalian systems.

(2) To prepare and isolate the four isomers of β-hydroxymethoxinine, β-hydroxyhomomethionine, β-hydroxymethionine, β-hydroxyaspartic acid, γ-fluroro- and γ-chloro-substituted β-hydroxyamino acids, and to prepare their N-acyl derivatives.

(3) To prepare the optically pure isomers of other amino acid analogs, such as those of β-2-thienylalanine, allylglycine, p-fluorophenylalanine, and mimosine (a tyrosine analog), and their N-acyl derivatives.

(4) To prepare the N-trifluoroacetyl derivatives of some of these β-hydroxy-amino acids, since the N-chloroacetylated derivatives of some of these compounds are not substrates of hog renal acylase while the corresponding N-triflouroacetyl derivatives are likely substrates of this enzyme.

(5) The optically pure diastereomers of the free acids as well as of the N-acyl derivatives will be tested for anti-tumor action in microbial anti-tumor screens, and when sufficient quantities are prepared, they will be tested against mammalian tumor systems.

Serial No. NCI-944(a)
1. Laboratory of Physiology
2. Energy Metabolism Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Total Metabolism

Previous Serial Number: Same

Principal Investigator: Seoras D. Morrison

Man Years:

Total:	2
Professional:	1
Others:	1

Project Description:

Objectives:

(a) Investigation of the patterns of heat production of rats with concomitant recording of feeding behavior and other activity with a view to finding how the energy expenditure, feeding and general behavior pattern are related to tumor growth and to finding the nature of the loads imposed by a tumor on the host.

(b) Investigation of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth.

(c) Examination of possible experimental methods of simulating in non-tumor-bearing rats the cachexia and decline of food intake found during neoplastic growth.

(d) Development of quantitative and reproducible assessment of behavioral output, to yield more sensitive and earlier detection of systemic effects of tumors.

Methods Employed: The methods of indirect calorimetry, operant conditional responses, placement of electrolytic lesions in or stimulation of the central nervous system, continuous or programmed infusions into unrestrained animals, and methods of orthodox nutritional studies. Ballistic methods of recording and partitioning total motor activity. Computer methods of numerical analysis of serial records of tumor growth and of continuous records of gaseous exchange. Sprague-Dawley rats and Buffalo rats are used in all animal experiments. Most tumor-bearing rats are inoculated with Walker 256 carcinoma, but other transplantable tumors are also used.

Major Findings:

(a and b) Part of the cachectic decline of food intake in some cancers is attributable to decline in effective energy flow of the host with resultant decline in energy available for motor activity (J. Nat. Cancer Inst. 51, 1535-1539). The response of components of water and energy exchange and of food intake to levels of salt loading showed that salt depletion of the host by the tumor is not a substantial cause of the fall in food intake or motor activity or of other abnormal energy exchange patterns in tumor growth (J. Nat. Cancer Inst., 1974 in press). This refutes previous reports on this point. Data processing programs are being developed for analysis of continuous metabolic data.

(c) Work in progress on feeding response to reduced environmental temperature indicates that this response, as well as that to nutrient density (annual report, 1972/73) is progressively attenuated with tumor growth. The persistent feeding deficits in animals recovered from lateral hypothalamic damage can be clearly discriminated from cachectic feeding failure - further evidence that abnormal hypothalamic function is now involved in cachexia. Preliminary reports on these topics were presented at Int. Conf. on Hunger and Thirst (Paris, Oct., 1973).

(d) The extent to which food intake is modified by change in amount of feeding activity and by change in feeding efficiency is being examined with respect to various normal influences on food intake and to changes with tumor growth and brain damage, separately and in combination. The tumor-bearer opposes the cachectic process by use of an active behavioral regulatory variant not so far found in non-cancerous states.

Significance for Cancer Research and the Program of the Institute: The findings on partition of energy expenditure and of tissue compartments and on processes influencing food and water intake make it possible to examine some features of tumor-host relationships that have not previously been subject to analysis. The methods and findings on sensitive and early changes in systemic response to tumors should be utilizable in refined animal testing of therapeutic agents. It is believed that the work is throwing useful light on the reasons for the rapid and extreme debilitation of the host during tumor growth. The section of the National Cancer Plan that the work most closely approximates is: Objective 6 (Develop the means to cure cancers and to retard the progress of cancers not cured). Approach 4 (Enhance the host's ability to eliminate or prevent further development of cancer).

Proposed Course of Project: Work on energy and water exchange of tumor bearers will be continued along with study on the interaction of metabolic and behavioral responses of normal and cancerous animals, with particular reference to the reasons for the changes in food and water intake and depletion of host tissues that occur during tumor growth. Objective 6, Approach 4.

Publications:

Morrison, S. D.: Differences between rat strains in metabolic activity and in control systems. Am. J. Physiol. 224: 1305-1308, 1973.

Morrison, S. D.: Limited capacity for motor activity as a cause for declining food intake in cancer. J. Nat. Cancer Inst. 51: 1535-1539, 1973.

Morrison, S.D.: Sodium and the cachexia and hypophagia of tumor growth. J. Nat. Cancer Inst., 1974. In press.

Serial No. NCI-919
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Urinary Excretion Patterns in Leukemia

Previous Serial Number: Same

Principal Investigator: James C. Reid

Man Years:

Total:	1
Professional	1
Others:	0

Project Description:

Objectives: This program is a developmental effort to expand the scope of urinary analysis. Its objectives are the development of a methodology to broaden greatly the chemical information which can be extracted from the urine and the evaluation of such information for potential utility from both the clinical and research standpoints. Experimentally, a search is being conducted for significant abnormalities in the excretion patterns of all detectable ultraviolet-absorbing components of a set of urine specimens collected from leukemic and control subjects. Such components include various nucleic acid congeners but are not limited thereto.

Methodology: The basic concept is that it may be feasible to construct an analytical system powerful enough to deal with the complexity of urinary composition by the application of a computational technique for multicomponent mixture analysis to urinary fractions obtained after preliminary chromatographic simplification. The data required are simply absorbance spectra, which can be obtained by a single non-specific instrumental procedure. The necessity for the application of many chemical procedures, each specific for a particular component, is eliminated thereby and the attendant reduction of labor would make it feasible to study many components simultaneously, i.e. to study patterns of excretion. Moreover, techniques which have been developed in this program permit quantitative estimation of the components without the necessity for chemical identification, a feature which facilitates further the recognition of pattern abnormalities if they should occur.

The preliminary chromatographic simplification is effected by a system of ion-exchange column procedures developed earlier in the course of this program. The system reduces each urine specimen to approximately fifty fractions. Each fraction is a mixture whose components typically number about a half-dozen, and in general the individual components can be obtained in small amounts by paper chromatography. The absorbance spectra of the isolated components then serve as the basis for computing the composition of the corresponding fraction obtained from other urinary specimens.

Status: It has become apparent that the computational procedures employed for the analysis of column-chromatographic profiles require numerical data more precise than those obtainable by the best paper-chromatographic procedures which could be devised for the isolation of pure specimens needed for the construction of a library of reference spectra. The limiting factors are paper blank, solvent blank, overlapping of spots and the low ratio of spot/background absorbance encountered in some instances. It is possible that these difficulties could be circumvented by heuristic computational approaches which would yield at least a partial analysis of the profiles and also that other spectrometric procedures might serve, such as laser-based or mass spectrometry. However, a major investment would be required for such investigations, which is not feasible under present circumstances. Accordingly the project is being terminated despite the attractiveness of the methodological concept, which is non-specific and could in principle be used for an automated examination of biochemical patterns in a wide variety of biomedical applications.

In briefest summary, the information obtained in the course of this work showed that the chromatographic profiles obtained from leukemic subjects exhibit a sparser pattern than those from controls, with a tendency to return toward the control pattern during remission. However, lacking detailed analysis of the peaks interpretations are purely subjective and the variations observed are not sufficiently clear-cut to yield useful conclusions.

Significance for Cancer Research and the Program of the Institute: National Cancer Plan, Objective 5 (diagnosis), Approach 4.

Future Course: Project terminated.

Serial No. NCI-936
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Recovery from effects of radiation or other initial treatment in terms of tolerance to subsequent treatments

Previous Serial Number: Same

Principal Investigator: Willie W. Smith

Man Years:

Total:	5
Professional:	1
Others:	4

Project Description:

Objectives: To study time and rate of recovery from effects of sublethal whole-body and abdominal irradiation in terms of altered tolerance to subsequent treatments.

Major Findings: It has been shown by others and confirmed by us that sublethal whole-body irradiation generally leaves a small amount of residual damage which reduces the tolerance to a subsequent exposure. One of our present objectives is to identify the site and nature of this damage. Mice given an initial whole-body irradiation and a challenging irradiation with their legs shielded 2 to 6 weeks later, showed survival equal to that of control mice given no prior irradiation. The "irreparable" residual damage thus appears to be associated with failure of complete hematopoietic rather than gastrointestinal recovery. With both marrow and gut exposed in the challenging irradiation the LD_{50} was approximately 20% lower than in mice given no previous exposure. On the other hand, when the marrow was shielded in the challenging irradiation, recovery appeared to be complete within 2 weeks.

Significance for Cancer Research and the Program of the Institute: There is increasing interest in therapeutic efficacy of combined treatment modalities, especially including radiation and chemotherapy. We have made several studies of this nature but digressed in order to gain a better understanding of the effects of repeated irradiation as specifically related to hematopoietic and gastrointestinal damage and recovery. We have made considerable progress in this effort and can soon profitably resume the studies of combined treatments.

Proposed Course of Project: The studies are being extended to include relationships between dose and residual damage in survivors of higher initial radiation in shielded and unshielded mice.

Publication:

Boggs, Sallie S., Wilson, Shirley M., and Smith, Willie W.: Effects of endotoxin on hematopoiesis in irradiated and nonirradiated W/W^v mice. Rad. Res. 56: 481-493, 1973.

Serial No. NCI-941
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunogenetic factors affecting bone marrow transplantation in irradiated mice: Modifying the functional capacity of bone marrow cells.

Previous Serial Number: Same

Principal Investigator: Delta E. Uphoff

Man Years:

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: To facilitate bone marrow transplantation as an adjunct to the administration of toxic levels of either irradiation or chemotherapeutic agents in the treatment of cancer. (6-1)

Methods Employed: Inbred mice, reciprocal F_1 hybrids, and especially derived substrains are exposed to lethally toxic doses of X-irradiation and engrafted with bone marrow cells from different donors to test the effect of different degrees of genetic disparity on survival. Characteristic changes in early survival patterns indicate changes in the ability of the marrow graft to respond to erythropoietic and antigenic stimuli while the long term survival is affected by the graft-versus-host reaction and alterations in leukemogenesis. The focus of attention is on the response of the grafted marrow against the recipient (GVH reaction) as the primary obstacle to clinical marrow transplantation. The present report deals primarily with immunogenetic factors influencing the severity of the GVH reaction.

Major Findings: The antigens controlled by the Y-chromosome of male mice are considered to be relatively weak antigens as compared with tissue antigens controlled by other genetic loci such as the histocompatibility-2 (H-2) locus. However, the GVH reaction produced in lethally irradiated inbred male mice by the engraftment of marrow from virgin or multiparous marrow donors of the same inbred (syngeneic) strain was as lethal or even showed greater lethality than that produced by marrow from unrelated (allogeneic) donors of the same H-2 genotype as the recipient. A survey of 13 inbred strains and substrains revealed that only 4 strains showed no evidence of a response by any females to male antigens; 5 strains gave no evidence that virgin females recognized the male antigens, however, engraftment of marrow from multiparous donors showed a significant reduction in survival indicating that these donors were sensitized to alloantigens of their male progeny; 4 strains showed a reduced survival from a GVH reaction following engraftment of marrow from virgin female donors and a greater reduction in survival following inoculation of maternal marrow. These varied results indicate that the male antigens are either controlled by multiple alleles at one or more loci or a single allele with a modified expression in different genetic backgrounds. Investigations of the response to male antigens has previously involved skin graft rejection which indicated that the expression of the male antigen (H-Y) was linked to specific H-2 genotypes, i.e., females of $H\text{-}2^b$ and $H\text{-}2^k$ strains rejected male skin while other strains did not. The marrow transplantation experiments, however, clearly show no association with this strong histocompatibility locus. In addition, the paradox that allogeneic marrow from an unrelated strain adapts to the foreign environment of the recipient while syngeneic female marrow does not adapt to the male environment became apparent. This difference may be a function of the recognition of the antigen or the processing of the antigen. More information on the presence of male antigens in other mammalian species is needed before maternal marrow should be considered for transplantation in male progeny in lieu of a compatible male sib.

Ova-transfer substrains in which homozygous fertilized ova are transplanted to the uterus of a foster mother were included in the investigation of response to the male antigen. These derived substrains showed survival patterns that differed from their ancestral strain. A comparison between the ancestral strain, the derived substrain and the strain of the ancestral foster mother revealed that either an increase or a decrease in responsiveness was transferred from the strain of the ancestral foster mother depending upon whether this strain was more or less responsive than the strain origin of the ova-transfer substrain. This shift in responsiveness of these substrains was similar to that reported previously using another test system involving a primary response toward H-2 antigens.

The investigation of the maternal influence on antigenicity and responsiveness of reciprocal hybrid mice was continued. The present approach differed in that marrow from reciprocal hybrid donors was transplanted into lethally irradiated recipients of the parental strains. Again the maternal influence affected the survival pattern. The hybrid cells apparently recognized the parental strain recipient as being quantitatively more antigenic than "self." In addition, the genetically identical reciprocal hybrid cells showed different degrees of response to the same parental strain recipient indicating that a qualitative difference in antigenicity was recognized by the hybrid cells, i.e., when the female of the hybrid cross is syngeneic with the recipient better survival occurrs than when the female is allogeneic with the recipient. An additive effect of a quantitative and qualitative difference in antigenicity was also demonstrated.

Significance for Cancer Research and the Program of the Institute: Bone marrow transplantation has furnished a very sensitive method of studying the antigen recognition system, degrees of responsiveness and maternal influences. The results of these experiments should have had a profound impact on basic immunologic concepts. However, there was a reluctance to accept these data because the system utilizes lethal doses of irradiation. The inertia prevalent for the last 10 years should now be overcome by the demonstration of degrees of antigenicity and degrees of responsiveness in reciprocal hybrids reported by Drs. Morse, Harrison and Asofsky (NIAID, LMI-111J) in the March 1974 issue of the Journal of Experimental Medicine. Their system employed a graft-versus-host reaction in new born mice and did not involve toxic levels of irradiation. Preliminary results on hybrid litters supplied from my breeding colony confirms my previous report of a maternal influence on responsiveness in the ova-transfer substrain RIIIeB. The full impact that these observations have on classical immunology will only be realized after reciprocal hybrids are more extensively used for immunologic investigation since presently accepted hypothesis will undoubtedly need to be revised.

Proposed Course of Project: No change in the course of this investigation is anticipated. Greater emphasis will be placed on the investigation of the response of F_1 hybrids to the parental strain as the experiments involving the male antigens are phased out. The H-2 specificity of the maternal modifications on ova-transfer substrains will be investigated as time and space permit. Other aspects of this project reported in previous years are being continued according to the most efficient utilization of the available mouse strains.

Publications:

Uphoff, D. E.: Maternal influence on mouse embryos and preservation of mutant strains by freezing. Science 181: 287-288, 1973.

Jacobs, B. B., and Uphoff, D. E.: Immunologic modification: Basic survival mechanism. Science (in press).

Uphoff, D. E.: Maternal influence on responsiveness of multiparous mice to the antigenic characteristics of their male progeny. J. Natl. Cancer Inst. (in press).

Serial No. NCI-942
1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Ionizing Radiation on Nucleic Acids, Proteins and Their Constituents: Radioprotective and Radiosensitizing Agents

Previous Serial Number: Same

Principal Investigator: Peter Riesz

Other Investigators:
S. Kominami, Visiting Fellow
V. Wee, Visiting Fellow

Man Years:

Total:	6.5
Professional:	3.0
Others:	3.5

Project Description:

Objectives: The effects of ionizing and ultraviolet radiation on biological macromolecules and their constituents are being investigated. For reproductive death, DNA is the target molecule in viruses and is at least a part of the target molecule in bacteria and mammalian cells. Radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals or by the "indirect effect" where radical species formed in the surrounding medium by radiation react with DNA. In the case of water, these species are hydrated electrons, hydrogen atoms and hydroxyl radicals. In the case of radiation damage to the chromosome, radicals formed in the nucleohistone may cause damage to the DNA.

In the chain of events that leads to loss of biological activity, free radicals play an important role. Thus, for example, when biologically active DNA of bacteriophage ϕ x 174 is irradiated in the dry state with γ-rays under vacuum, cysteamine protects to the same extent (by about a factor of 1.6) against inactivation and the production of free radicals. We have found recently that the addition of certain metal ions to DNA in the ratio of 1 metal ion per 10 nucleotides, can reduce the radical yield by a factor of about 10.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity of these macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

Methods Employed: When dry proteins or nucleic acids are irradiated with gamma rays or ultraviolet radiation in the absence of air (at 10^{-4} mm pressure), trapped free radicals are generated. Subsequent exposure to tritiated hydrogen sulfide or hydrogen iodide leads to the formation of carbon-tritium bonds. After removal of exchangeable tritium the protein is hydrolyzed, the amino acids are separated on an automatic amino acid analyzer and the tritium content of each amino acid is determined by means of an associated flow counter. It has been shown that the tritium distribution so obtained corresponds closely to the free radical distribution at the time of exposure to the tritiated free radical interceptor.

Trapped radicals in lyophilized proteins and nucleic acids were produced by:

(a) Gamma-radiolysis using a Co^{60} source.

(b) Photolysis with 2537 Å light using a low pressure Mercury arc.

A PCQ-X1 Photochemical Lamp with 4 circular lamps was used; 80-90% of the total radiation is at 2537 Å.

Electron Spin Resonance studies were carried out at Walter Reed Army Medical Center using the Varian E-9 ESR Spectrometer located in the laboratory of Dr. E. S. Copeland.

Proteins and DNA were tritium labeled on exchangeable sites by exposing the dried materials to tritiated water vapor (5 curie/gm) at 20 torr for 10 minutes in a high vacuum system located in a hood in the Isotope Laboratory (Bldg. 21).

Major Findings:

1. Effect of Metal Ions on the Formation of Strand Breaks and Radicals, and on Hydrogen Transfer in γ-Irradiated DNA in the Solid State (with V. Wee, S. Kominami and H. Moss)

The radiation-induced formation of strand breaks in DNA *in vivo* has been observed in many systems and the existence of genetically controlled repair systems for these lesions has been demonstrated. Double strand breaks in particular are considered to play an important role in cell death. Strand breakage in DNA in solution has been investigated by several groups. The present work deals with strand breaks produced in the solid state. For every strand break produced in the γ-radiolysis of DNA a monophosphate ester is produced from which inorganic phosphate can be released by treatment with alkaline phosphatase. The formation of inorganic phosphate can be followed by a sensitive colorimetric assay, and hence the yield of strand breaks can be measured as a function of dose.

The effects of metal ions on the formation of strand breaks by γ-radiolysis of calf thymus DNA in the solid state at 298° K. in the absence of oxygen were investigated. The G-value (number of strand breaks per 100 electron volts absorbed by DNA) for DNA is 1.6 and is decreased by a factor of 5 for chromic ions, by a factor of 3 for cupric ions, and by about 2 for nickel and cadmium ions. No effect was observed for magnesium ions. Similar effects of metal ions on the formation of carbon radicals in γ-irradiated DNA at 77° K. and 298° K. were measured by electron spin resonance and were also observed during the transfer of tritium from exchangeable to carbon-bound sites in γ-irradiated DNA at 298° K. Our work has shown that the metal ions exert their protective effects at an early stage of γ-radiolysis prior to stabilization of the radicals at 77° K. The similarity of the effects of metal ions on strand breaks, carbon radicals and hydrogen transfer could be explained by assuming that the metal ions decrease the yield of a common precursor species.

2. Effects of Metal Ions on Chain Scission and Radical Formation in Collagen and Polyadenylic Acid (with F. Friedberg and A. K. N. Nandetkar, Biochemistry Dept., Howard University, and S. Kominami)

When lathrytic collagen (Av. M. Wt. 118,000) and polyadenylic acid (Av. M. Wt. 218,000) are γ-irradiated in the solid state in the absence of O_2, the average molecular weight is decreased, since chain scission predominates over crosslinking. Globular protein denature and aggregate but do not split into smaller units under the same conditions.

Changes in average molecular weight were measured by the ultracentrifugal "low speed" sedimentation equilibrium method. The percentage decrease of the Weight Average Molecular Weight of lathrytic collagen irradiated to 25 Megarads was 72%. In the presence of metal ions, the percentage decreases were 0.9% for cupric ions (1 metal ion per 10 amino acid residues), 28% for ferrous ions (1:10), 49% for nickel ions (1:10, 70% for nickel ions (1:100) and no effect for cadmium ions (1:10). Molecular weight changes were measured in the dose range of 15-50 Megarads for collagen and polyadenylic acid. Electron spin resonance studies on the effects of metal ions on the formation of carbon radicals at 298° K. were carried out in the .1-15 Megarad range.

Similar protective effects of metal ions ($Cu^{2+} > Fe^{2+} > Ni^{2+} > Cd^{2+} \sim$ DNA) were observed for chain scission and radical formation at room temperature. The sequence of events which leads to chain scission is known in more detail for polypeptides than for polynucleotides. From the ESR studies of Henriksen and Saxebol, it follows that the cation radicals produced at 77° K. by γ-radiolysis of peptides can decarboxylate at higher temperatures, while the anion radicals may deaminate, thus leading to chain scission. These cation and anion radicals are also precursors of the neutral radicals produced at room temperature. Hence, if metal ions prevent the formation of the cation or anion radicals, similar effects on chain scission and radical formation at room temperature are to be expected.

In the case of polyadenylic acid, similar effects of metal ions on chain scission and radical production at room temperature were also found. However, at present, no direct ESR evidence exists which connects the cation and anion radicals produced at the earliest stages of irradiation with the formation of chain breaks.

3. Effects of Metal Ions on the γ-Radiolysis of Polycrystalline Djenkolic Acid (with S. Kominami)

Gamma-radiolysis of polycrystalline djenkolic acid at room temperature produces two groups of carbon radicals. The radicals of group A either decay by a second-order reaction or are converted to sulfur radicals by a pseudo first-order process, while the radicals of group B can only be converted to sulfur radicals. The carbon radicals of groups A and B appear to be the same chemical species but differ in their microenvironments. Addition of Co^{2+}, Ni^{2+} and Fe^{2+} at a ratio of 1 metal ion per 10 molecules of djenkolic acid decreases the initial yield of carbon radicals and increases the second-order decay rate and the percentage of carbon radicals belonging to group A. Addition of Na_2SO_4, $NaNO_3$, $CdSO_4$ and Ni^{2+}-EDTA has no effect. The second-order decay may be explained by a vacancy-controlled bulk-diffusion process; metal ions appear to exert their influence by increasing the number of lattice defects. Addition of certain metals, particularly Cd^{2+} and Ce^{4+}, decreases the rate of conversion of the carbon radicals of group A to sulfur radicals. Ni^{2+} and Ce^{4+} ions also prevent the radiation-induced destruction of sulfur radicals. The effects of metal ions on a variety of different processes can thus be differentiated in γ-irradiated djenkolic acid.

4. ESR and X-Ray Diffraction Studies of Copper-Doped Single Crystals of Cystine [with S. Kominami; J. Silverton and T. Akiyama (LC, NHLI)]

Previous work by Kominami has shown that γ-irradiation at 77° K. of single cyrstals of cystine hydrochloride in the absence of light and O_2, produces cation radicals, anion radicals and neutral radicals. At certain orientations of the single crystal to the static magnetic field of the spectrometer, the ESR spectra of the three species are separated from one another. Hence, it seemed likely that cystine hydrochloride might afford a unique opportunity for studying the effect of small amounts of cupric ion on the formation of the initial radical species produced by γ-radiation. After many months of work, large copper-doped crystals of cystine hydrochloride suitable for ESR studies were obtained. The crystals of the copper-doped cystine hydrochloride are monoclinic with space group $P2_1$ and contain 2 water molecules per cystine, while those of cystine hydrochloride are monoclinic with space group C2 and contain no water. The positions of all of the atoms in the unit cell were determined by x-ray diffraction and the structure was refined to a final r value of 0.051.

Preliminary ESR studies on the cupric binding site in the cystine hydrochloride crystal showed that the cupric ions were located in definite positions in the unit cell. The future course of this work is to obtain a detailed identification of the cupric binding sites, to determine the role of the cupric ions in decreasing the free radical yield and to evaluate the contribution of the water molecules in the radiation chemistry of the copper-doped cystine hydrochloride crystal.

Significance for Cancer Research and the Program of the Institute: Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy, (2) carcinogenesis, (3) stability of the genetic pool, (4) the suppression of the immune mechanism, and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained. Objective 6, Approach 1.

Proposed Course of Project: To continue studies on the effects of ionizing radiation on macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents will be investigated.

Honors:

Member of Editorial Board (Radiation Research Society). Nominated to be candidate for Councilor for Chemistry of the Radiation Research Society.

Publications:

Rotlevi, E., Moss, H. M., Kominami, S., and Riesz, P.: Effects of metal ions on radical formation in γ-irradiated DNA in the solid state. Proc. NY Acad. Sci. 222: 387-410, 1973.

Kominami, S. and Riesz P.: Effects of metal ions on the γ-radiolysis of polycrystalline djenkolic acid. Radiation Res. (in press)

LABORATORY OF PATHOPHYSIOLOGY
SUMMARY STATEMENT
ANNUAL REPORT
July 1, 1973 through June 30, 1974

The primary objective of the Laboratory of Pathophysiology is the understanding of conditions which determine arrest and regulation of growth in neoplastic tissues. Ten projects are presently carried on with this major objective in mind:

1) N-nitrosomethylurea-induced Mammary Carcinomas. The goal of this project was the induction of a mammary carcinoma which could mimic the human counterpart in most of its biological properties. Available mouse tumor models metastasize but are not hormone responsive; rat models are hormone responsive but do not metastasize. Using N-nitrosomethylurea we were able to devise a treatment which induces within 3 months mammary carcinomas in 90% of Buffalo/N inbred female rats. The tumors are hormone responsive, metastasize consistently and induce hypercalcemia, thus mimicing the human counterpart almost completely. This model will be used routinely not only in the Laboratory of Pathophysiology but also in the NCI Breast Cancer Task Force program.

2) Role of RNA during Tumor Regression. Regression of rat mammary carcinomas is induced in vivo by depriving the host of the appropriate hormone. Our previous work indicated that a burst of RNA synthesis occurred as one of the first events observed after hormonal deprivation, followed by synthesis of lysosomal enzymes. In order to see whether these events were indispensable for regression to occur, several drugs were used to block in vivo more than 90% of RNA and protein synthesis. Despite this blockage, tumor regression proceeded unimpaired. Probably cell death is induced by events still unknown and the lysosomal enzymes help only to digest the residues. A study of the fate of tumor RNA species during regression revealed that one RNA molecular species is continuously produced despite the block of more than 90% of RNA synthesis. Work is in progress to define this molecule and to ascertain whether its synthesis is essential for regression to occur.

3) Reproduction in vitro of Hormone-dependent Tumor Regression. The complexity of the in vivo model system by hormonal deprivation of the host makes difficult a separate analysis of each component of the regression process. We need an in vitro system in which neoplastic cell populations can be grown in tissue-like structures without interference of host factors, and can be induced to regress, as in vivo. The development of the capillary culture unit has enabled us to grow neoplastic mammary epithelial cells (human and rodent) to tissue-like structures on artificial capillaries. Now we are attempting to reproduce the in vitro regression process by hormone deprivation.

4) Role of Cyclic AMP in Tumor Regression. Since the hypothesis of cyclic AMP as a second messenger has received some confirmatory evidence, it appeared reasonable to attempt to influence tumor growth by changing the in vivo cyclic AMP level. Work in this direction has thus far yielded the following information: a) Derivatives of cyclic AMP do inhibit growth of some mammary carcinomas and hepatomas of the rat. b) Growth inhibition is dose dependent

and reversible upon cessation of treatment. c) After a single cyclic AMP injection a sharp increase in activity and quantity of acid RNase is observed, exactly as in tumors regressing after hormonal withdrawal. d) Two cell populations have been isolated from Walker 256 mammary carcinoma and 5123 hepatocarcinoma, respectively. One cell population rapidly regresses but the other continues to grow under dibutyryl-cyclic AMP treatment. The same behaviour was observed after prostaglandin E_1 treatment. Thus, a promising biochemical tool has been found for studying cyclic AMP-dependent regression by comparing responsive and unresponsive cell populations from the same tumor. e) A simple increase of cyclic AMP level in the tumor is insufficient to produce regression; something else, though unknown at this time, must happen during cyclic AMP treatment. Studies in this area will be extended to characterize both quantity and quality of cyclic AMP binding-protein and to determine its relationship with protein kinase activity. The possible relationship between cyclic AMP and hormone-induced tumor regression will also be investigated.

5) Role of Poly-ADP-ribose in DNA Replication. Synthesis of poly-ADP-ribose occurs during the S- and G2- phase of the cell cycle. As cells enter mitosis, synthesis falls to about one-third. Arrest of cell division can be obtained in vitro by addition of nicotinamide, a product of the polymerization reaction catalyzed by poly-ADP-ribose synthetase. The activity of this enzyme(s) peaks in G_2 cells, coincidental with maximal labeling of the polymer and can be blocked by inhibitors of protein synthesis. There is enough circumstantial evidence to favor some regulatory function of poly-ADP-ribose in cell division. The need for the polymer synthesis is not clear, and the effects of its degradation on the sequence of events in the cell cycle are also unknown. Both areas will be investigated further with the objective of detecting whether poly-ADP-ribose may be an efficient tool for interfering in the tumor growth process.

6) Role of Neoformation and Destruction of Vessels in Growth and Regression of Mammary Tumors. Answers to 2 questions were sought: Are "preneoplastic" lesions able to induce angiogenesis, and can this ability be utilized to predict neoplastic transformation before other signs are evident? Based on the postulation that a humoral factor (tumor angiogenesis factor) is responsible for the neovascularization of growing tumors, carcinomas, normal mouse mammary tissue and hyperplastic alveolar nodules of $C_3H\text{-}A^{vy}$ mouse mammary gland were transplanted on the iris of rabbit eyes. Angiogenesis was studied daily by direct observation with slit lamp microscopy and fluorescein angiography. It was found that new formation of vessels was elicited by 97% of carcinomas, 30% of hyperplastic nodules, and 2% of normal mammary transplants. Induction of neovascularization in 2 lines of hyperplastic alveolar nodules (obtained from Dr. D. Medina) of low and high tumor potential was 30% and 80% respectively. Thus the ability to induce vascularization appears to be the only presently known marker of "preneoplasia" unrelated to cell division. Acquisition of the ability to induce vascularization may be a property which defines neoplastic transformation independently from changes in the regulation of cell growth.

7) In Vitro Culture of Human Vascular Endothelium and Smooth Muscle Cells. As a side-project of the angiogenesis study, an attempt was made to develop cultures of human endothelium to be used for *in vitro* assay of the tumor angiogenesis factor. Endothelium was obtained from the vein of umbilical cords, treated immediately after delivery with collagenase and trypsin. By an appropriate combination of digestion procedures, it has been possible to obtain pure endothelial cell cultures, as identified in EM by the Weibel-Palade body, and pure smooth muscle cultures, as identified by the large cell size and ability to elaborate extracellular material such as basement laminae and elastic microfibrils. A most interesting observation was the capacity of these cultures to accumulate intracellular lipids and undergo morphological changes similar to those observed in atherosclerotic plaques *in vivo*. NHLI investigators interested in atherosclerosis have been informed of this finding.

8) Loss of Tumor Cells in the Efferent Blood. The study of this problem was considered important for two main reasons: to establish whether loss of cells via blood was a substantial component of tumor shrinkage during regression and to evaluate a possible relationship between the level of cell shedding into the circulation and metastasis formation. The work was done using MTW9 rat mammary carcinoma because we could obtain an ascites form of this tumor and use the washed cells to produce an antiserum. From the tissue isolated preparation of the tumor, previously developed in this laboratory, one can collect blood coming out of the whole tumor for several hours. In this blood the neoplastic cells were counted using an immunofluorescent procedure. Growing MTW9 mammary carcinomas lost, on the average, $5 \cdot 10^6$ cells/day/g; during regression the cell loss was equal or less. This input of neoplastic cells into the circulation corresponds to at least a daily inoculum sufficient to transplant the tumor. Nevertheless, MTW9 does not metastasize.

9) Fluid loss from Growing and Regressing Tumors. As a side project of the study on neoplastic cell loss via tumor efferent blood, the fluid loss by the tumor surface was estimated. It is known that neoplastic tissues do not have an anatomically demonstrable lymphatic net and also that a halo of edema is often present around a tumor mass. Work done in our laboratory in collaboration with the Institute of Chemical Engineering, University of Delaware, suggested that convective mass transport in the interstitial spaces of neoplastic tissue should occur. Now we have indeed been able to show that 5 to 10% of the plasma entering the tumor leaves not through the vessels but at the tumor surface in the form of convective currents spreading through the tumor parenchyma.

10) Expanded Application of the Capillary Culture Units. A group of collaborative projects is being carried on to expand the application of the capillary culture unit to problems outside the area of our immediate concern but the solutions of which appear to us both feasible and particularly urgent.

a) Virus production: The capillary culture unit allows long term culture of a cell population organized in tissue-like structures and under ideal conditions to produce viruses continuously. Attempts are being made to develop an artificial lymphatic system within the culture, so that virus particles can be drained out of the intercellular spaces without disturbing the microenvironment of the culture. (Collaboration with Dr. P. Qasba).

b) Culture of human bone marrow. Bone marrow dedifferentiates rapidly in standard cultures because special macromolecules required to promote differentiation cannot be preserved after each change of media. We assumed that in capillary culture units these molecules should not diffuse through the capillary wall; therefore, very small amounts of them present in the pericellular fluid should be sufficient to maintain bone marrow differentiation for long periods. The supposition proved correct and presently all cell types of the bone marrow can be cultivated. This has never been obtained before and could represent a breakthrough in bone marrow cultivation if further data sustain the preliminary results. (Collaboration with Dr. M. J. Cline).

c) Production of urokinase by human fetal kidney. Urokinase, used in the treatment of thrombosis, is presently produced from human kidney cells maintained in monolayer cultures. The technique is cumbersome and complicated and the product very expensive. In the past we observed that human choriocarcinoma cells maintained in capillary culture units produced 10-fold more gonadotropin than in monolayer cultures. We are attempting to cultivate in the same way human kidney cells, hoping to obtain the same high yield from cultures which should last weeks and require only 10% the effort needed to maintain monolayers. Thus far, we do not have results. (Collaboration with Dr. J. T. Pisano).

Serial No. NCI-8202
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: N-nitrosomethylurea-induced Mammary Carcinomas: A Model which Mimics Post-menopausal Human Breast Cancer.

Previous Serial No.: None

Principal Investigaror: P.M. Gullino

Other Investigators: H.M. Pettigrew

Cooperating Units: Biometry Branch, NCI

Man years:

Total: 2
Professional: 1 1/2
Other: 1

Project Description:

Objectives: To develop a mammary carcinoma in the rat which mimics the clinical and pathological characteristics of the post-menopausal breast cancer of humans.

Methods Employed: N-nitrosomethylurea was given intravenously in 3 injections of 5 mg/100 g BW each, the 2nd at 4 and the 3rd at 8 weeks after the first injection. Buffalo/N inbred females were used since they were found most responsive to the treatment. Standard techniques of analysis of tumor incidence, histology, hormone responsiveness, transplantability etc. were employed.

Major Findings: 1) A method able to produce 90% incidence of mammary carcinomas was found and is now operative on a routine basis. 2) Incidence of tumors was 90% in Buffalo/N ♀, 60% in Sprague-Dawley ♀ and 36% in Fisher 344 ♀. 3) In Buffalo/N the biological properties of tumors were as follows: castration before treatment prevented oncogenesis; castration after treatment reduced the total number of tumors per rat but not the incidence; castration after the tumor was established produced growth arrest or temporary regression. Metastasis to bone marrow and spleen were constant, to the liver, frequent, and to the lungs, rare. After removal of the primary tumor, metastases continued to grow. Almost all tumors were transplantable intraperitoneally and/or subcutaneously in the inguinal region of intact as well as ovariectomized and adrenolectomized animals. Transplanted tumors were able to metastasize as the primary ones. When the tumor mass of each was 15 g or larger, hypercalcemia was usually observed. Doubling times of NMU primary and transplanted carcinomas were about 7 days. Cachexia ensued at the 5th week from the onset of the first tumor.

The NMU-induced tumors in rats castrated at the end of treatment appear to be the only model available to mimic the post-menopausal mammary carcinoma of humans.

Significance for Cancer Research (NCP Objective 4 Approach 1 and 6-3): The model is necessary to study the human tumor.

Proposed Course of Research: To complete the study within 1974.

Honors and Awards: see NCI-328

Publications: None

Serial No. NCI-368
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Mechanisms of Mammary Tumor Regression

Previous Serial No. : Same

Principal Investigator: P.M. Gullino

Other Investigators: None

Cooperating Units: None

Man years:
Total: 1 1/2
Professional: 1/2
Other: 1

Project Description:

Objectives: To understand the mechanism of regression in hormone-responsive mammary tumors.

Methods Employed: Tumor regression is obtained by castration, in primary tumors induced by 7-12-DMBA and N-nitrosomethylurea. In transplantable MTW9 regression is obtained by removal of the mammotropin source, usually MtTW10 pituitary tumor. Standard techniques are used such as sampling of tumor interstitial fluid by micropore chambers, continuous infusion of radioactive substances through the aorta, separation of nuclear and various cytoplasmic fractions, gel electrophoresis of RNA extracted from the fractions etc.

Major Findings: 1) RNA and protein synthesis has been inhibited *in vivo* and the treatment of the animal has been arranged as to produce growth arrest of the tumor for one week or more. Regression of these growth-arrested tumors has been induced by hormone deprivation and the events occurring in the regressing tissue are being studied when new formation of RNA and protein is blocked. The rationale is that as regression ensues, fragments of dead cells are cannibalized by neighboring tumor cells which increase their metabolic activity, thus overshadowing the events which trigger regression. 2) Tumors in growth arrest induced by inhibitors of RNA and protein synthesis, regress rapidly when hormonal deprivation of the host is produced. During regression under such conditions certain RNA molecular species are synthesized at a higher level than before regression. Work is in progress to define the nature of these molecules and to understand whether their synthesis is necessary for regression to occur.

Significance to Cancer Research (NCP Objective 6, Approach 3): If we understand the events occurring during regression of mammary tumors induced by hormonal deprivation, we should be able to produce tumor regression.

Proposed Course of Research: To continue to study the mechanism of mammary tumor regression.

Honors and Awards:

1. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. I. U.S.Patent Application Serial No. 254,678, April 1972.
2. Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. II. U.S. Patent Application.
3. Gullino, P.M., Grantham, F.H., Hill, D.M., and Rowland, J.: Cage for continuous infusion of radioactive compounds and drugs into small laboratory animals. U.S. Patent Application pending.

Publications:

1. Gullino, P.M., Cho-Chung, Y.S., Losonczy, I., and Grantham, F.H.: Increase of of RNA synthesis during mammary tumor regression. Cancer Res. 34: 751-757, 1974.

Serial No. NCI-5219
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In Vitro Simulation of Hormone-dependent Mammary Tumor Regression

Previous Serial No.: Same

Principal Investigator: R.A. Knazek

Other Investigators: P.M. Gullino

Cooperating Units: None

Man Years:

Total: 2 + 1 Stride
Professional: 1/2
Other: 1 1/2 + 1 Stride

Project Description:

Objective: To reproduce and study in vitro the hormone-dependent regression process of mammary tumor observed in vivo.

Methods Employed: A bundle of tube-shaped semi-permeable membranes will simulate the in vivo capillary bed. Isolated cells injected into the space surrounding these artificial capillaries settle onto and adhere to the capillary surfaces. When such a capillary bundle is perfused with tissue culture medium, the cells receive nutrients and have by-products removed by diffusion through the capillary wall. The MTW9 rat mammary carcinoma will grow in vivo only when a prolactin/growth hormone-producing pituitary tumor is present in the host animal; the mammary tumor will regress rapidly when the pituitary tumor is removed. The artificial capillary culture technique (described above) is being used to grow both of these solid tumors in vitro. Two capillary culture units are arranged in series, with the upstream unit containing pituitary tumor cells and the downstream unit containing the target mammary carcinoma. The latter cells are then stimulated by the secretory products of the pituitary cells as the medium flows through both capillary culture units, mimicking the in vivo stiuation.

Major Findings: Pituitary tumor cells form solid tumor masses within the artificial capillary bed and produce significant quantities of prolactin. Initial observations indicate that the mammary tumors are also growing.

Significance to Cancer Research (NCP Objective 4 Approach 1): The environment which artificial capillaries provide is more physiologic than that of any other type of cell culture. This allows isolated cells to form solid tumor masses *in vitro* and to exhibit behavior that more closely resembles that of the *in vivo* state. Thus, solid mammary tumors can be formed *in vitro* and the hormone-dependent regression process can be studied without uncontrollable *in vivo* factors.

Knowledge of the effect of various hormones on both the growth and regression processes may reveal the mechanisms involved and perhaps provide information that can be used to induce clinical improvement of patients with breast cancer.

Proposed Course of Research: The effect of prolactin, growth hormone, insulin, estrogen, and glucocorticoids upon the behavior of the MTW9 tumor will be studied using the artificial capillary culture technique.

Honors and Awards:

1) Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. I. U.S. Patent Application Serial No. 254,678, April 1972.

2) Knazek, R.A., Gullino, P.M., Dedrick, R.L., and Kidwell, W.R.: Cell culture on semi-permeable tubular membranes. II. U.S. Patent Application.

3. Knazek, R.A.: Visiting faculty member at the W. Alton Jones Cell Science Center "Perfusion Culture Systems", Lake Placid, N.Y. May 16-17, 1974

Publications:

1. Knazek, R.A., Kohler, P.O., and Gullino, P.M.: Hormone production by cells grown *in vitro* on artificial capillaries. *Exptl. Cell Res*. 84: 251, 1974

2. Knazek, R.A.: Solid tissue masses formed *in vitro* from cells cultured on artificial capillaries. *Fed. Proc*., 1974, *In Press*.

Serial No. NCI-5216
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Role of Cyclic Nucleotides on In Vivo Tumor Growth Inhibition

Previous Serial No.: Same

Principal Investigator: Yoon Sang Cho-Chung

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

Objectives: Previously our studies demonstrated a significant increase in several lysosomal enzyme activities and quantities during the early phase of hormone-dependent mammary tumor regression. We now believe that this increase is a consequence not the cause of the regression process. In an attempt to define the triggering mechanism of mammary tumor regression, we investigated the role of cyclic nucleotides on tumor growth in vivo (control of cell growth in vitro by these nucleotides has been shown).

Methods Employed:

1) Primary, 7,12-DMBA-induced and transplantable MTW9 rat mammary tumors (hormone dependent), primary, nitrosomethylurea-induced mammary carcinoma (hormone-responsive), Walker 256 carcinoma (hormone-independent mammary tumor), hepatoma 5123 and fibrosarcoma (CSE TB 206) were used.

2) Cyclic nucleotide injections (8 mg/0.2 ml NaCl/day/rat s.c.) were initiated when tumor size was about 3 g. Tumor growth was expressed as percent change in volume calculated from the formula, 4/3 $\pi r3$, where r is 1/2 the average of the longest and shortest diameters measured daily.

3) Acid RNase was assayed by a modification of the method of Kalnitsky et al and enzyme quantitation was performed by single radial immunodiffusion with crystallized acid RNase as reference. Glucose-6-phosphate dehydrogenase was

assayed by both spectrophotometric and histochemical methods. cAMP levels in tumors were measured by Gilman's competitive protein binding assay.

Major Findings:

1) cAMP derivatives, DBcAMP, 8-thiomethyl-cAMP and 8-bromo-cAMP inhibited in vivo growth of rat tumors: primary, 7,12-DMBA-induced, transplanted MTW9 and Walker 256 mammary carcinomas, and hepatoma 5123. The same nucleotide had little inhibitory effect on the growth of nitrosomethylurea-induced mammary tumors and did not affect fibrosarcoma.

2) Growth inhibition was dose dependent and reversible upon cessation of treatment.

3) Serum estrogen concentration did not change during DBcAMP treatment in the host bearing hormone-dependent tumors.

4) In growth arrested MTW9, a significant early increase of acid RNase activity and quantity was found following DBcAMP treatment and the increase was shown to be due to new synthesis of the enzyme, utilizing an immunochemical method; precipitation of in vivo labeled enzyme with monospecific anti-acid RNase antiserum produced against crystallized enzyme.

5) Treatment with DBcAMP resulted in the disappearance of tumor microsomal glucose-6-phosphate dehydrogenase (isozyme 3) but not liver enzyme of a similarly treated rat; growth of tumor was arrested while hepatectomized liver regenerated as in untreated controls (during DBcAMP treatment).

6) Pulsed ^{3}H-thymidine incorporation into DNA indicated that inhibition of DNA synthesis is apparent only at later time following DBcAMP treatment.

7) Two cell populations have been isolated from W256 and hepatoma 5123 by selective transplantation: one rapidly regresses (DBcAMP-responsive) and the other rapidly grows (DBcAMP-unresponsive) under DBcAMP treatment.

8) Prostaglandin E_1 induced regression of only the responsive W256 when injected (1 mg/day/rat) into rats bearing both responsive and unresponsive tumors.

9) 6 days of DBcAMP treatment resulted in 2-3-fold increase of intracellular cAMP concentration over basal levels and a slight increase of cAMP-phosphodiesterase activity in both responsive and unresponsive tumors.

Significance to Cancer Research (NCP Objective 6 Approach 3): These studies contribute to the understanding of the biochemical mechanisms underlying the regulation of normal and abnormal growth and will offer the means of inhibiting the growth of experimental tumors which mimic human cancers. The lack of any correlation between in vivo tumor growth inhibition and elevation of intracellular cAMP concentration implies that intracellular cAMP level is not the sole determinant of DBcAMP-induced growth arrest.

Proposed Course of Research: To extend studies on the mechanism of cAMP action on tumor growth inhibition, the following proposal is made:

1) Characterize DBcAMP-responsiveness in terms of tumor cAMP-binding protein both quantitatively and qualitatively. Purification of cAMP-binding protein is necessary for the ultimate results.

2) Determine the activity of cAMP-activivated protein kinase in tumors and correlate with cAMP-binding protein activity.

3) Determine whether the regulatory subunit of protein kinase is the cAMP-binding protein in tumors.

4) Determine whether cAMP action is at the translational or transcriptional level and correlate results with nuclear histone.

5) Determine whether or not the triggering mechanism of hormone-dependent mammary tumor regression, induced by hormonal deprivation, is related to the cAMP system.

Honors and Awards: None

Publications:

1. Cho-Chung, Y.S. and Gullino, P.M.: Mammary tumor regression: V. Role of acid ribonuclease and cathepsin. J. Biol. Chem. 248: 4743-4749, 1973.

2. Cho-Chung, Y.S. and Gullino, P.M.: Mammary tumor regression: VI. Synthesis and degradation of acid ribonuclease. J. Biol. Chem. 248: 4750-4755, 1973.

3. Cho-Chung, Y.S. and Gullino, P.M.: In vivo inhibition of growth of two hormone-dependent mammary tumors by dibutyryl cyclic adenosine 3',5'-monophosphate. Science 183: 87-88, 1974.

4. Cho-Chung, Y.S. and Gullino, P.M.: Effect of dibutyryl cyclic adenosine 3',5'-monophosphate on in vivo growth of Walker 256 carcinoma: Isolation of responsive and unresponsive cell populations. J. Natl. Cancer Inst., 1974, In press.

Serial No. NCI-5211
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Initiation and Maturation Phenomenon in the Replication of DNA

Previous Serial No.: NCI-5211

Principal Investigator: William R. Kidwell

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 3
Professional: 1
Other: 2

Project Description:

Objectives: Progression of the cell through the cell cycle is characterized by a series of events which precede one another in ordered sequence. How is this ordered sequence maintained? It is probable that at the completion of one stage of the cell cycle the signal for the succeeding stage is triggered. The objective of this investigation is to characterize the mechanism by which the ordered sequence of cell cycle events is controlled. Specifically, the investigation is focused on the synthesis of poly (ADP-ribose) which occurs in S and in the G_2 period which follows. The mechanisms by which the synthesis of the polymer is altered (increased, decreased) and the influence of polymer synthesis and/or degradation on subsequent cell cycle events is to be considered.

Methods Employed:

Cell Synchrony: Cells were accumulated in G_1 by growth to high densities in monolayer cultures. After trypsinization, cells were arrested at G_1/S by treatment with hydroxyurea. Resumption of progression to cell division was initiated by replacement of the blocking medium with inhibitor-free medium.

Poly(ADP-ribose) Synthesis in Vitro: Synthetase activity was assessed on the basis of incorporation of 8-^{3}H-adenosine labeled NAD into an acid insoluble form, utilizing purified nuclei as an enzyme source.

Isolation of Poly(ADP-ribose) Synthesized in Vivo: Following pulse labelling with 2-H^3-adenosine, nuclei were isolated and solubilized in 0.5% SDS. Proteins were removed by pronase digestion and phenol extraction. Samples were heat denatured then chromatographed on benzoylated, naphthoylated DEAE columns. Single stranded RNA and DNA contaminants were strongly absorbed to the BND-column matrix while poly(ADP-ribose) was recovered in 0.3 and 1.0 M NaCl washes. Repetition of the procedure through one additional column gave a purified product that was identified as poly(ADP-ribose) on the basis of column chromatography of material rendered acid soluble following venom diesterase treatment.

Major Findings:

Synthesis of Poly(ADP-ribose) in Intact Cells: Previously it was shown that poly(ADP-ribose) was synthesized in S-phase cells but much less synthesis was observed in G_1 cells. These studies have been extended and labeling of the polymer has also been detected in G_2. Maximal labeling of poly(ADP-ribose) with H^3-adenosine occurs about 2 hours after termination of the S-phase. As cells enter mitosis, synthesis falls to about 1/3 of the G_2 level. Pulse-chase studies indicate that poly(ADP-ribose) turns over with a half-life of about 1-2 hours.

Coincident Inhibition of Cell Division and Poly(ADP-ribose) Synthesis: Poly (ADP-ribose) synthesis is inhibited in vivo and in vitro by nicotinamide, a product of the polymerization reaction catalyzed by poly(ADP-ribose) synthetase. Nicotinamide (20mM) added to cell cultures that have just completed S-Phase, fail to divide. Nicotinamide treatment reduces the incorporation of H^3-adenosine into poly(ADP-ribose) in G_2 cells by 50-60%. Compared to control cultures, the mitotic index is reduced by 90%.

While a causal relationship between these two processes is not established, the results suggest that poly(ADP-ribose) synthesis is a necessary prerequisite for the mitotic events which follow G_2.

Changes in Poly(ADP-ribose) Synthetase During the Cell Cycle: Increased poly(ADP-ribose) synthesis during S-phase does not correlate with the amount of synthetase activity as measured in isolated nuclei. Rather, there is an inverse relationship between polymer synthesis and the levels of chromatin associated poly(ADP-ribose) glycohydrolase which degrades the polymer. In contrast, poly(ADP-ribose) synthetase activity peaks in G_2 cells coincident with maximal labeling of the polymer in intact G_2 cells. The increase in the levels of poly(ADP-ribose) synthetase which occurs as cells progress from S into G_2 can be blocked by agents which inhibit protein, RNA or DNA synthesis.

Significance to Cancer Research: (NCP Objective 6, Approach 3) Understanding the mechanisms whereby cell progression through the cell cycle is controlled may suggest ways in which cancer cells can be reversed to a non-transformed state. A model system for coupled regulation of cell progression is characterized by (1) the event occurring at a specific time in the cell cycle (G2, for instance); (2) for this event to occur, completion of a preceding cycle event (S-phase) is a prerequisite; (3) blocking the event of G_2 leads to a failure of the cell to traverse through the next event (mitosis). Poly(ADP-ribose) synthesis during G_2 fits the category of coupled regulation. Hydroxyurea added before the end of S-phase arrests cells in S and also blocks the increase in poly(ADP-ribose) synthetase activity which normally occurs in G_2. Blocking the synthesis of poly(ADP-ribose) in G_2 is correlated with the cells' failure to progress to mitosis.

Proposed Course: The major objective of the coming year will be to determine the regulatory function of poly(ADP-ribose). It has been noted that following polymer synthesis, there is a rapid turnover. In the S-phase degradation is probably catalyzed by a glycohydrolase while in late G_2 and mitosis, it may be phosphodiesterases of cytoplasm since the nuclear membrane is being disassembled. These catabolic enzymes produce different degradation products. Are these degradation products acting as signals triggering the next event of the cell cycle? This possibility is to be explored in two ways: (1) determining whether blocking poly(ADP-ribose) breakdown interferes with progression of the cell cycle; (2) assessing whether the two different catabolic products induce premature advancement of cells to a succeeding stage of the cell cycle. Also, it is clear that the nature of the poly(ADP-ribose) synthetase and proteins which serve as acceptors for ADP-ribose need to be characterized. We will be attempting to purify the synthetase for this purpose by standard fractionation procedures including affinity chromatography.

Honors and Awards: None

Publications:

1. Colyer, R.A., Burdette, K.E., and Kidwell, W.R.: Poly(ADP-ribose) synthesis and DNA replication in synchronized mouse L-cells. Biochem. Biophys. Res. Commun. 53: 960-966, 1973.

2. Kidwell, W.R. and Colyer, R.A.: Poly(ADP-ribose) synthesis during the cell cycle. Proc. of the Fogarty International Center. 1974 In Press.

3. Wittes, R.E. and Kidwell, W.R.: A kinetic approach to the determination of the S-phase pool size of thymidine triphosphate in exponentially growing mouse L-cells. J. Mol. Biol. 78: 473-486, 1973.

Serial No. NCI-8201
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanisms of Tumor Neovascularization

Previous Serial Number: Same

Principal Investigators: M.A. Gimbrone, P.M. Gullino

Other Investigators: R.S. Cotran, C. Handenschild

Cooperating Units: Departments of Surgery and Pathology, Harvard Medical School and Childrens Hospital Medical Center, Boston, Mass.

Man Years:

Total: 2 1/2
Professional: 2
Others: 1/2

Project Description:

Objectives: This project is concerned with 2 basic aspects of tumor neovascularization:

1) Growth in in vitro culture systems of the 2 major cell components of the blood vessel wall: endothelium and smooth muscle; and determining the effect of tumor cells and TAF extracts directly on these cell types.

2) Studying the angiogenic capacity of normal, premalignant and malignant tissues to induce neovascularization in an intraocular implantation model, to determine whether this biological ability is correlated with malignant potential.

Methods Employed:

1) Human endothelial cells and vascular smooth muscle cells are isolated from umbilical cord vessels by enzymatic perfusion techniques and cultured in vitro. Mitogenic effects of tumor cell extracts and other test materials are assayed by ^{3}H-thymidine incorporation. Growth behavior of both cell types are studied by phase contrast and electron microscopy.

2) Biopsies of mammary tissues from high tumor incidence strains (C^3H, $C^3H\text{-}A^{vy}$) are implanted intraocularly in NZW rabbits. Neovascular responses

are studied by direct in vivo slit-lamp microscopy and fluorescein angiography, and histologically in ^{3}H-thymidine autoradiographs.

Major Findings:

1) Techniques have been developed which permit the in vitro culture of human vascular endothelium and smooth muscle as separate homogeneous cell populations for the first time. Identity of each cell type has been established by ultrastructural analysis. Endothelial cell cultures have been shown to behave as density-dependent populations with respect to DNA synthesis. Tumor cell extracts (TAF) with demonstrable angiogenic activity in in vivo test models, do not stimulate DNA synthesis in this in vitro target cell system.

2) Biopsies of malignant mammary tissues consistently elicit a neovascular response when implanted intraocularly in NZW rabbits. The morphology and time course of new vessel outgrowth is similar to that observed with implants of homologous rabbit tumors. Normal mammary tissue biopsies rarely cause any detectable vascular changes. Biopsies of premalignant lesions, hyperplastic alveolar nodules (HAN), often induce neovascularization comparable in degree to frankly cancerous lesions, in the absence of any histological evidence of overt malignant transformation. Biopsies of HAN outgrowth lines with a high potential for malignant progression produced neovascularization with significantly ($p<.001$) more frequency than HAN-lines with a low malignant potential.

Significance for Cancer Research (NCP Objective 4, Approach 3, and Objective 5, Approach 2):

1) The failure of in vivo "active" TAF to induce endothelial cell DNA synthesis directly in vitro, if substantiated, implies that certain undetermined host factors (cellular or humoral) may be important intermediaries in tumor-induced neovascularization in vivo.

2) The observation that premalignant mammary lesions can induce neovascularization in proportion to their ultimate malignant potential may provide a new definition for the "preneoplastic state". If the mechanism of stimulation of neovascularization by premalignant lesions is the same as with malignant tumors, then the demonstration of neovascularization in an appropriate test system could be used to predict ultimate cancer risk of an atypical tissue biopsy.

Honors and Awards: Guest lecturer, Department of Pathology, Harvard Medical School, Boston, Mass., December 1973.

Publications:

1. Gimbrone, M.A., Cotran, R.S. and Folkman, J.: Human vascular endothelial cells in culture: Growth and DNA synthesis. J. Cell Biol. 60: 673-684, 1974.

2. Gimbrone, M.A., Cotran, R.S., Leapman, S., and Folkman, J.: Tumor growth and neovascularization: an experimental model using the rabbit cornea. J. Natl. Cancer Inst. 52: 413-427, 1974.

Serial No. NCI-8203
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In Vitro Culture of Human Vascular Endothelium and Smooth Muscle Cells: Ultrastructure and Biosynthetic Properties

Previous Serial No.: None

Principal Investigator: M.A. Gimbrone, Jr.

Other Investigators: R.S. Cotran

Cooperating Units: Mallary Institute of Pathology, Harvard Medical School, Boston, Mass.

Man Years:

Total: 1 1/2
Professional: 1
Others: 1/2

Project Description:

Objectives:

1) Study the cytologic and ultrastructural properties of cultured human endothelial and smooth muscle cells to determine structural criteria for their identification and morphologic features accompanying their rapid growth in vitro.

2) Study the extracellular amorphous matrix and formed elements synthesized and secreted by the 2 major cellular components of the blood vessel wall.

Methods Employed:

1) Mixed primary cultures of endothelium and vascular smooth muscle are obtained by enzymatic perfusion of human umbilical cord vessels. Endothelial and smooth muscle cells are selectively subcultured as homogeneous populations by varying trypsin-harvest parameters and culture medium properties.

2) Phase contrast and stained cytologic preparations are examined at varying stages of culture growth. Extensive transmission electron microscopy of thin-sectioned, Epon-embedded material is also performed.

Major Findings:

1) Human endothelial cells can be identified in culture by the presence of the Weibel-Palade granule which has a characteristic ultrastructural appearance. This morphologic feature was uniformly observed in endothelium at all stages in culture and was totally absent from smooth muscle of the same vessel wall.

2) Smooth muscle cells elaborate significant amounts of extracellular material, including amorphous basement lamina, elastic microfibrils and mature (banded) collagen fibrils. In older cultures, they also accumulate intracellular lipid and undergo morphological changes similar to those observed in the development of atherosclerotic plaques *in vivo*.

Significance for Cancer Research (NCP Objective 4 Approach 3):

1) Little is known of the growth behavior and synthetic properties of the cellular elements of host blood vessels which proliferate in the course of tumor-induced neovascularization. The isolation of endothelium and smooth muscle as separate, homogeneous populations *in vitro* will allow analysis of these activities.

2. The occurrence of atherosclerotic-like lesions in human smooth muscle cultures is a new observation, worthy of further investigation, beyond the NCP outline.

Honors and Awards: None

Publications:

1. Gimbrone, M.A., Cotran, R.S. and Folkman, J.: Endothelial regeneration: Studies with human endothelial cell in culture. *Ser. Haematol*. 4: 453-455, 1973.

Serial No. NCI-8200
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Tumor Cells in Tumor-efferent Blood

Previous Serial Number: Same

Principal Investigatro: T.P. Butler

Other Investigator: P.M. Gullino

Cooperating Units: None

Man Years:

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objective: Identification of tumor cells in efferent tumor blood with subsequent quantitation of tumor cell shedding by growing and regressing tumors.

Methods Employed: MTW9 hormone-dependent rat mammary tumors have been grown in rats and isolated in vivo so that they were served by a single artery and vein. Under anesthesia the efferent vein was cannulated and the entire efferent blood of the tumor was collected over time intervals from 30 minutes to 3 hours. Simultaneous blood samples were obtained from the jugular vein and carotid artery. Hematocrit and total and differential nucleated cell counts were performed on all samples, both for growing and regressing tumors.

Antisera to the tumors were induced in New Zealand rabbits by intravenous and subcutaneous injections of single cell suspensions of ascites tumor. Rabbit anti-tumor globulin was prepared by ammonium sulfate precipitation, and absorption was carried out against normal rat white blood cells, marrow cells, and liver cells. Indirect immunofluorescence, employing fluorescein isothiocyanate-labelled caprine anti-rabbit gamma globulin, was used to detect and quantitate tumor cells in aliquots of efferent blood from growing and regressing tumors.

Major Findings: Tumor cells can be distinguished from non-tumor cells in the blood and can be counted. The number of tumor cells shed into blood per 24 hours per gram tumor (roughly 5 x 10^6 cells) is relatively constant whether the tumor is growing or regressing. The number of whole cells shed constitutes about 10% or less of estimated total cell loss of growing and regressing tumors. The number of cells shed in 24 hours is sufficient for a tumor transplant inoculum, but metastases are never seen. (Proc.Am.Assoc.Cancer Res. 15: 46, 1974 - Abstract)

Significance for Cancer Research (NCP Objective 4 Approach 2):

1) A quantitation of the number of cells lost by the tumor is necessary for assessing whether tumor regression takes place by cell shedding or cell dissolution within the tumor. 2) Metastasizing tumors differ from non-metastasizing tumors with respect to the number of neoplastic cells released in the general circulation; 3) Immunologic tolerance is related to the number of neoplastic cells released in the general circulation.

Proposed Course of Research: Publication of results; project to be terminated June 30, 1974.

Honors and Awards: None

Publications: None

Serial No. NCI-8204
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Net Fluid Flow into Interstitial Space from Tumor Capillaries

Previous Serial No.: None

Principal Investigator: T.P. Butler

Other Investigators: P.M. Gullino

Cooperating Units: None

Man Years:

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objective: Determination of net fluid flow into interstitial space of growing and regressing tumors.

Methods Employed: MTW9 hormone-dependent rat mammary tumors have been grown in rats and isolated in vivo so that they were served by a single artery and vein. Rat erythrocytes were labeled with ^{51}Cr and infused into MTW9 hormone-dependent tumor-bearing rats. Efferent blood from the tumor was collected for 1 hour in 10 minute serial aliquots. Arterial blood samples were simultaneously collected at 10 minute intervals. Hematocrits and total and differential white counts were done on all samples, from both growing and 48-hour-regressing tumors. Radioactivity of paired efferent and arterial samples were measured by gamma counting and compared. Using the rate of efferent blood flow and the degree of hemoconcentration detected by arterio-venous differences in hematocrit and radioactivity, the rate of net loss of fluid from tumor vessels into interstitial space was calculated.

Major Findings: Hemoconcentration takes place within the tumor as shown by significant and consistent arterio-venous differences in hematocrits and white cell counts. Preliminary results with ^{51}Cr appear to confirm the intratumor hemoconcentration detected by the hematocrits. Calculation of the rate of fluid loss from tumor vessels into interstitial space has been made.

Significance to Cancer Research (NCP Objective 6 Approach 1): Quantitation of the net fluid flow into tumor interstitial fluid is necessary 1) to elucidate the process of transport of nutrients and metabolic waste products through poorly vascular tumor tissue; 2) to assess possibilities of altering intratumor transport of nutrients, waste products, or anti-neoplastic agents to alter tumor growth.

Proposed Course of Research: Publication of results; project to be terminated June 30, 1974.

Honors and Awards: None

Publications: None

Serial No. NCI-8205
1. Laboratory of Pathophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Application of the Capillary Culture Technique to (a) Virus Productions, (b) Bone Marrow Culture, and (c) Urokinase Synthesis.

Previous Serial No.: None

Principal Investigator: (a) R.A. Knazek, W.R. Kidwell, P. Qasba, and P.M. Gullino
(b) R.A. Knazek and M.J. Cline
(c) R.A. Knazek and J.J. Pisano

Other Investigators: None

Cooperating Units: (a) Laboratory of Biochemistry, NCI
(b) Hematology Division, Department of Medicine, UCLA
(c) Experimental Therapeutics Branch, NHLI

Man Years:

Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives: (a) Development of a method that provides continuous and/or improved production of virus particles *in vitro*. (b) Growth and study of normal bone marrow *in vitro*. (c) Development of a method to provide continuous production of large quantities of human urokinase.

Methods Employed: (a) Established or primary mammalian cell suspensions are injected into the extracapillary space of an artificial capillary culture unit. When the capillaries are perfused with tissue culture medium these cells grow to multilayer depths at which time they are infected with a small inoculum of virus. The viral progeny are removed from the extracapillary space at set times during the subsequent weeks of culture. (b) Aspirates of normal bone marrow are injected into the extracapillary space of an artificial capillary culture unit. The capillaries are then perfused with oxygenated tissue culture medium which provides a continuous source of nutrients and enables cell product removal by diffusion without disturbing the pericellular environment of the extracapillary space. Stimulatory macromolecules of high molecular weight are inoculated with the cell suspension but do not diffuse through the capillary walls. Thus, the cells are provided with a high stimulatory concentration of poietic factor and a large nutrient/product reservoir. (c) Urokinase is currently used clinically in the treatment of thrombotic phenomenon. This enzyme is obtained commercially from human fetal kidney cells (HFK) maintained in monolayer forms requiring rather cumbersome and expensive techniques. This project consists of culturing HFK cells within the artificial capillary culture units to high cell densities and collecting urokinase as it is secreted into the perfusion medium.

Major Findings: (a) Initial observations indicate that SV-40 can be produced within Vero cells grown in the artificial capillary system. (b) Granulocytes, monocytes, erythrocytes and megakaryocytes may all be maintained in the artificial capillary culture units. Initial studies indicate that both white and red cell lines can divide, grow, and respond to their respective stimulatory factors within the artificial capillary network. The morphology of the various cell types appears normal after two weeks of perfusion, the longest time period tried to date, in contrast to other techniques that exhibit rapid macrophage overgrowth. This is the first time that megakaryocytes have been maintained in vitro. (c) No HFK cell growth was observed when artificial capillaries permeable to 10,000 MW substances were used. No urokinase was detected in the perfusate medium.

Significance to Cancer Research (NCP Objective 3 Approach 3): (a) This technique should provide a convenient and efficient method for producing large quantities of virus for both study and vaccine production. (b) All blood cell components may now be maintained in vitro. Study of their metabolism and growth characteristics will provide further insight into the behavioral controls of the hematopoietic system, both in normal and neoplastic states. The possibility also exists for a patient's bone marrow to be aspirated and maintained or grown in vitro to serve as a tissue replacement source following reinjection after chemotherapy has depressed its marrow. Neoplastic marrow may also be maintained in a nearly physiologic condition to study its response to different chemotherapeutic agents and programs. (c) Development of a technique that permits diploid cells to remain differentiated both morphologically and functionally will be a valuable tool for studies on transformation, differentiation, and dedifferentiation.

Proposed Course of Research: (a) Different viruses and cell types will be studied using several different types of capillary culture units to improve the yield and convenience of the technique. (b) Capillaries of various types and configurations will be studied to provide optimum conditions for cell growth and observation of various hematopoietic components. (c) A new artificial capillary, permeable to substances up to 300,000 MW has been developed at our request. This highly permeable membrane may permit maintenance and growth of normal human differentiated cells in vitro. A variety of these capillaries are now under study.

Honors and Awards: None

Publications: None

LABORATORY OF THEORETICAL BIOLOGY

SUMMARY

July 1, 1973 - June 30, 1974

Summary Report

The activities of the Laboratory of Theoretical Biology (LTB) fall into several areas: the biophysics of membranes, developmental biology, kinetic-metabolic systems and mathematical and computational methodology and theory related to modeling. Most of the work is theoretical, although some experiments are also carried out in the laboratory. A number of the modeling activities are in collaboration with experimental groups both at NIH and elsewhere.

Most of the work on membranes is carried out by Dr. Blumenthal. He is interested in membrane models from the point of view of transport, structural organization and cooperativity. In connection with this he has developed some theories to explain cellular transports and concentrations on the basis of control mechanisms at the membrane level. These theories were applicable to metabolites transported across or located within membranes. The theories can also be extended to study dynamic arrangements of membrane phospholipids.

Physical mechanisms for ion transport across the membrane are also being investigated by Dr. Blumenthai. Using ionophoric material, isolated from $(Na^{+}+K^{+})$ ATPase containing electroplax membranes in a bilayer membrane system, pore formations have been detected from fluctuating discrete conductance levels. This provides new insights into the nature of the membrane pores and their kinetics.

Another problem related to membranes is the modeling of the mechanism related to adenylate cyclase activity (Rendell, Berman). This is a collaborative study with Dr. Rodbell's group in NIAMDD and involves liver and adipose-tissue cell membranes. A model has been proposed involving an activated and inactivated state of the enzyme, and a catalytic site that interacts with the substrate (ATP) and an inhibitor (HATP). This model has been tested and found compatible with the data, and equilibrium constants for these states have been determined. The modeling of the regulating sites (GTP and hormone) is now in progress.

Analysis of mechanisms underlying determinations and transdeterminations in drosophila is an experimental project carried out by Dr. Kauffman in an effort to verify (and to extend) a theory he has put forth on control circuits in developmental biology. According to this theory, the determination of wings, legs, antennas, etc. from imaginal discs in drosophila are controlled by a circuit composed of at least four bistable states (for example, leg = 1010, wing - 1111, etc.). A transdetermination is a chance switch of one or more circuits from one state to another. Dr. Kauffman is presently trying to fuse cells from different imaginal discs hoping thereby to determine the dominance of the various circuits.

Several systems are being actively modeled by this group, usually in collaboration with one or more experimental groups at NIH or elsewhere. The modeling of metabolic systems has readed now a high level of complexity and sophistication and on-hand experience in dealing with large systems provides new insights into the theory and methodology of modeling as related to large systems. This activity also serves as a pilot project in establishing model-systems for several important metabolic systems in man - a task too complex at present for experimentalists.

The lipoprotein system (Berman, Hall) is modeled in collaboration with Dr. Levy's group (NHLI), Dr. Malmendier (University of Brussels) and Dr. Fisher (University of Florida). The model previously developed for apoB proteins using an exdogenous label (Dr. Fisher) has been adapted in part for the apoB and apoC protein studies carried out by Dr. Levy's group. A more complete and extensive model has evolved now that covers apoB and apoC kinetics in very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins. This model was tested for normals and for various types of abnormals (type I to V, Fredrickson classification) and showed new important features for some of the abnormals. In all hyperproteinemias the process of delipidation is slowed down. A new degradation pathway has been discovered for all abnormals. Perhaps most important - the model permitted, for the first time, the characterization of the cause of the type III abnormality. Our model suggests that it is due mainly to an overproduction of VLDL which, in turn, probably saturates the delipidating enzyme systems and forces delipidation through an alternate pathway - causing abnormal VLDL particles.

Complementary to the apo-protein studies are Dr. Malmendier's experiments on triglyceride kinetics in VLDL, LDL and HDL. These support in part the results obtained with the apo-proteins but reveal some new features previously not detected. This modeling, however, is still in progress - too early to give results.

In collaboration with the Gerontology Research Center of the NICHD (Drs. Andres, McGuire) we (Berman) have been studying the kinetics of glucose and insulin, the control of insulin on glucose metabolism and the control of glucose on insulin secretion in man, under normal and abnormal conditions. A model for the insulin and glucose subsystems has been developed and control sites and modes of control of insulin on glucose have previously been identified. A model for the control of glucose on the secretion of insulin has now also been developed and tested. The complete system is being evaluated with respect to stability and response to various idealized perturbations.

In collaboration with Dr. Dubois of NIAMDD, the modeling of stomach emptying and secretion mechanisms is continued. Previously a tentative model was developed for the dog. The dog studies have been extended to cover a wider test range for the variables (acidity, volume) and studies have also been initiated for humans.

Other metabolic studies carried out over the year include lithium kinetics and the effect on iodine metabolism (Berman, McGuire), pertechnetate (Berman), methotrexate (Berman, Millar), heme biosynthesis (Millar, Tschudy).

Work is progressing on the SAAM computer program and supporting activities (Berman, Weiss, Chu, McGuire). New extensions have been added to SAAM (Weiss) and new numerical analysis procedures have been introduced to permit the modeling of more complex systems (Chu). A conversational SAAM capability has been implemented on the PDP-10 computer at NIH (McGuire). The remote terminal software has been improved in reliability and new capabilities have been added for communication with two computers (Bidinger).

Serial No. NCI-8300
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: SAAM Computer System

Previous Serial Number: Same

Principal Investigator: Mones Berman

Other Investigators: Marjory F. Weiss
Robert McGuire
Sherwood Chu

Man Years:

Total:	1.3
Professional:	1.3
Others:	0

Project Description:

Objectives: To develop a general prupose computer program for modeling biokinetic systems that may readily be used by investigators not sophisticated in mathematics or programming. Initiated in 1959, the program continues to be expanded and revised as new features are added.

Major Findings: A major revision of the structure of the program was undertaken and completed to permit additional features to be added to the program. Greater efficiency in operation and greater ease of use were additional results of this revision. New features have permitted the solution of some problems not previously within the scope of the SAAM program (Weiss, Chu).

A conversational version of SAAM for the PDP-10 computer at NIH has been implemented (R. McGuire).

A revision of the SAAM Manual has been partially completed and will describe the newest version of the program.

Significance for Cancer Research: The SAAM computer program has been a most essential tool in the modeling of various systems described elsewhere in this Laboratory report. Its wide use in other centers in the United States and elsewhere is further evidence of its value in biomedical research. It is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Serial No. NCI-8300
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Proposed Course: Broadening of the scope of the program, its use as a conversational tool and refinement of mathematical and statistical methods will continue.

Serial No. NCI-8301
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Modeling Theory and Applications

Previous Serial Number: Same

Principal Investigator: Mones Berman

Other Investigators: Robert McGuire (NCI)
Paul Van Eerdewegh (NCI)
Elizabeth A. McGuire (NICHD)
Reubin Andres (NICHD)
Andre Dubois (NIAMDD)
Margaret Hays, VA Hospital Buffalo, N.Y.

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives: Analysis of data on metabolic systems, and further development of modeling theory and techniques through the applications of the SAAM computer program.

Major Findings: Some initial qualitative criteria have been worked out for describing models and data and for testing compatibility between the two (Van Eerdewegh).

The glucose-insulin feedback control system has been further defined for man. A model for the control of glucose on the secretion of insulin has been proposed and behavior of the complete feed-back system is being investigated.

A model has been proposed and tested for the action of lithium on the peripheral metabolism of thyroid hormone in man (R. McGuire).

Studies on the stomach emptying and secretion processes in man have been initiated and are presently being modelled (with Dr. Dubois, NIAMDD).

Serial No. NCI-8301
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance to Cancer Research: The methodology of modeling is most essential for studying the behavior of complex systems for both normal and abnormal states. This most closely fits into Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: This is a continuing process for the development of theory for and the application of modeling techniques.

Publications:

Dubois, A. and Berman, M.: Gastric emptying and secretion, kinetic analysis and physiological model. Proceedings of the Fourth International Symposium on Gastrointestinal Motility. 1974. In press.

Sherwin, R.S., Krammer, K.J., Tobin, J.D., Insel, P.A., Liljenquist, J.E., Berman, M. and Andres R.: A model of the kinetics of insulin in man. J.C.I., May, 1974. In press.

Insel, P.A., Kramer, K.J., Sherwin, R.S., Liljenquist, J.E., Tobin, J.D., Andres, R. and Berman, M.: Modeling the insulin-glucose system in man. Federation Proceedings, July, 1974. In press.

Serial No. NCI-8302
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Computational Mathematics

Previous Serial Number: Same

Principal Investigator: Sherwood C. Chu

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To evaluate, develop and implement mathematical techniques used in the modeling and simulation of biological systems. This entails the development of efficient numerical procedures and their computational implementation.

Methods Employed: The primary mathematical tools needed to evaluate or develop an algorithm are from the theories of differential equations, numerical analysis, and linear algebra. Computer programming provides the means for implementation.

Major Findings: A numerical procedure for the solution of initial value problems for general systems of ordinary differential equations has been developed. It has been implemented and incorporated into the SAAM program for routine usage. This algorithm is far less time consuming, although less accurate, than the method that was previously used.

In conjunction with Mrs. M. F. Weiss, a substantial addition is being made to the SAAM program in order to incorporate new capabilities to aid in modeling and simulation.

Significance for Cancer Research: Sound computational techniques facilitate and permit the mathematical analysis of complex biological systems; it, thus, is consistent with Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: The analysis of the various mathematical aspects of biological modeling will be continued. An effort will be made to broaden the applicability of existing algorithms to more general systems as well as making them more computationally efficient.

Serial No. NCI-8303
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Ion Transport Through Modified Lipid Bilayer Membranes

Previous Serial Number: NCI-8303

Principal Investigator: Dr. Robert Blumenthal

Cooperating Units: Laboratory of Biophysics, NINDS, NIH
Laboratory of Neurochemistry, NINDS, NIH
Dept. of Radiation Biology and Biophysics
University of Rochester School of Medicine and Dentistry
Rochester, N.Y. 14623

Man Years:

Total:	.5
Professional:	.5
Others:	0

Project Description:

Objectives: To study the physical mechanisms for ion transport in synthetic membranes. To develop the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell membranes. The ultimate goal of the synthetic membrane studies is the elucidation of the permeability gating mechanism of membrane receptors.

Methods Employed: The bilayer membranes are formed from natural membrane extracts, oxidized cholesterol or pure lipids in an aperture between two electrolyte solutions. The electrical properties of the membranes are measured before and after application of an activating factor. Measurement of electrical fluctuations provide information about the molecular rearrangements of ionophoric material in the membrane. The study of active transport involves similar measurements on ionophoric material isolated from a tryptic digest of the Na^+-K^+ ATPase active transport system of electric eel.

Major Findings: With the ionophoric material isolated from (Na^++K^+) ATPase containing electroplax membranes we established pore formation by observation of fluctuating discrete conductance levels. Electrokinetic studies on pore formation and ion selectivity measurements provided insight into the permeation mechanism of the ionophoric material.

Serial No. NCI-8303
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance to Biomedical Research and the Program of the Institute: The gated permeability properties of doped bilayer bears many similarities to those of natural excitable membranes. The analysis of single conducting channels has given support to the idea of discrete conducting channels in membranes. With respect to cancer, possible regulatory functions of action potentials and ion gating include cell division, parthenogenic activation of ova, pseudopod formation and gating of cell junctions. The bilayer has proven to be a sensitive probe for the molecular properties of ion conducting channels in membranes. The extension of the bilayer techniques as an assay for ionophores isolated from the $Na^{+}+K^{+}$ ATPase active transport system offers the promise of the reconstitution of the active transport process. Ion pumping is of particular importance in the control of cellular functions. Objective 3, Approach 5 of the National Cancer Plan.

Proposed Course of the Project: Experiments with the peptide fragments isolated from electroplax are in progress to find conditions where ion selectivity and gating by means of ATP are maintained. We hope to extend the bilayer technique to the study of receptor-transmitter (acetylcholine, norepinephrin), receptor-hormone (glucagon, insulin) and receptor-antibody systems.

Publication:

Ehrenstein, G., Blumenthal, R., Latorre, R. and Lecar, H.: Kinetics of the opening and closing of individual EIM channels in a lipid bilayer. J. Gen Physiol., 1974, in press.

Serial No. NCI-8304
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Dynamic Membrane Transport Models

Previous Serial Number: NCI-8304

Principal Investigator: Dr. Robert Blumenthal

Man Years:

Total:	.5
Professional:	.5
Others:	0

Project Description:

Objectives: To develop models describing transformations of levels of cellular activity induced by transient environmental stimuli at the membrane level.

Methods Employed: Expressions are derived for the transient and steady state concentration levels of metabolite on the membrane surface. Simulations are carried out making use of the PDP-10 of the central NIH computer facility.

Major Findings: The oligomeric membrane transport model developed last year to account for instabilities, oscillations and chemical wave propagation of metabolites has been extended to the dynamic arrangement of membrane phospholipids.

Significance to Biomedical Research and the Program of the Institute: The significance may be listed in terms of the major findings of the formal models: 1) Biological examples of multiple steady states include transitions of respiratory states in mitochondria, of uptake of metabolites in transformed cells and of levels of inducer in differentiating cells. 2) Sustained oscillatory behaviour of cells may account for a time basis for development e.g., slime mold amoeba send out periodic waves of cyclic AMP upon aggregation and morphogenesis. 3) Pattern formation of molecules on the membrane surface may account for the dynamic architecture of membranes. The models may specifically be applied to transformations of arrangements of membrane components on the membrane surface.

This project most closely supports Objective 4, Approach 4 of the "National Cancer Plan" by developing mathematical models of the cancerous process.

Serial No. NCI-8304
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Proposed Course of Study: The models will be further developed in relation to biological data on dynamic membrane phenomena.

Honors and Awards: Discussant at a Neurosciences Research Program Conference on Functional Linkage in Biomolecular Systems.

Publications:

Blumenthal, R. P.: Dynamic patterns in active transport. Israel J. Chem., 11: 341-355, 1973.

Katchalsky, A., Rowland, V., and Blumenthal, R. P.: Dynamic patterns in brain cell assemblies. Neurosciences Res. Prog. Bull., 12:Vol I, 1974.

Serial No. NCI-8305
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Remote Communications Computer System

Previous Serial Number: Same

Principal Investigator: Peter A. Bidinger

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To develop a remote terminal computer operating system which will interface with various other computers and utilize various on-line input-output devices.

Major Findings: The Univac 1004 emulator has been expanded to (a) simultaneously drive two communication lines, (b) handle punch output, and (c) handle disk and magnetic tape devices. The DOS operating system has been modified to handle (1) an x-y Plotter, (2) a paper tape spooler-reader, and (3) a video-jet printer.

Publication:

Bidinger, P.A. and Berman, M.: The design and implementation of a mini-computer system as an enhanced multi-line remote terminal. Proceedings of the 13th Annual Technical Symposium of the Washington, D.C. Chapter of the ACM and the National Bureau of Standards. In press.

Serial No. NCI-8306
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Kinetic Modeling of Human Plasma Lipoprotein Metabolism

Principal Investigator: Mones Berman

Other Investigator: Marshall Hall, III

Cooperating Units: Molecular Disease Branch, NHLI
(Dr. R. Levy)
University of Florida, Gainsville
(Dr. W. Fisher)
University of Brussels, Belgium
(Dr. C. Malmendier)

Man Years:

Total: 1.2
Professional: 1.2
Other: 0

Project Description:

Objectives: To develop a qualitative and quantitative understanding of lipoprotein metabolism in man and to identify abnormalities through the modeling of lipoprotein kinetics. Because of the need for diverse, extensive data on a variety of patients, collaboration with several experimental groups was established.

Methods Employed: Mathematical modeling is the tool used for the integration and analysis of the data. This is performed with the help of the SAAM computer modeling program.

Major Findings: The apoprotein C system has been studied most extensively. It reinforced and refined the model developed previously for the apoB lipoproteins and the role of High Density Lipoproteins (HDL) in the kinetics of apoC. Major findings are 1) ApoC proteins are synthesized and first enter plasma with HDL. 2) There is a reduction in delipodation rate of VLDL for all hyperlipoproteinemias, suggesting saturation in the delipidation rates. 3) The abnormality in type III hyperlipoproteinemia is due to an overproduction in VLDL, a saturation of the normal VLDL delipodation mechanism and the opening of a bypass delipodation pathway that generated abnormal β-mobility particles.

Serial No. NCI-8306
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance for Cancer Research: Modeling becomes important in trying to isolate the abnormality responsible in hyperlipemics, given a very complicated system with complicated interactions. In the long run, it is hoped that by isolating the abnormality, treatment can be geared to the abnormal site identified. Clinically, this is relevant to atherosclerosis, cardiovascular disease, and other metabolic disorders. Objective 4, Approach 4 of the National Cancer Plan.

Proposed Course: The results obtained with protein kinetics will be integrated with experiments involving other lipoprotein moieties, such as the triglycerides.

Publication:

Malmendier, C.L., Delcroix, C. and Berman, M.: Interrelations in the oxidative metabolism of free fatty acids, glucose and glycerol in normal and hyperlipemic patients. J.C.I., 1974. In press.

Serial No. NCI- 8312
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Tital: Computer Applications: Programming and Data Processing

Previous Serial Number: None

Principal Investigator: Florence K. Millar

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To facilitate the investigation of biomedical research problems through the application of computer programming and computer data processing.

Methods Employed: Use is made of the PDP-11/20 computer of this laboratory, the National Bureau of Standards Univac 1108 computer, and the central NIH computer facility. The projects reported may be collaborative efforts with investigators in other units, as shown below.

Major Findings:

Computer Applications:

(1) Processing and plotting of data from liquid scintillation spectrometers:

Comparison of isoaccepting species of tRNA isolated from normal and malignant cells. (Dr. J. F. Mushinski, C/LBGY).

(2) Analysis of enzyme kinetics:

Kinetic studies of UMP-CMP kinase and on uridine-cytidine kinase (Dr. B. P. Anderson, Mr. A. S. Liacouras, C/LPHY; Dr. T. Q. Garvey,III, Mass. General Hospital.) Use is made of programs developed by Cleland (Nature 198:463, 1963) to handle the reaction equations.

Serial No. NCI-8312
1. Laboratory of Theoretical Biology
2. Mathematical BIology Section
3. Bethesda, Maryland

Transfer of Operations from the IBM-1620 Computer to the PDP-11/20:

Utility programs have been written to facilitate use of hardware components added to the original PDP-11/20 system (plotter, paper tape reader, printer).

Modelling and Simulation with the SAAM Computer Program:

(1) The analysis of carbon radical decay curves following the production of radicals by gamma radiation of djenkolic acid. (S. Kominami, P. Riesz, C/LPHY).

(2) Simulation of heme biosynthesis by a kinetic model of feedback control mechanisms. (D. P. Tschudy, C/MET).

(3) Modelling of the kinetics of methotrexate in man. (P. J. Creaven, VA Hospital, Washington, D.C.).
Modifications to the model proposed earlier (see NCI-8309 FY'73) are being explored.

Significance to Biomedical Research and the Program of the Institute: Computer programming applications contribute to the investigation of research problems by providing (1) accurate and efficient methods for handling laborious calculations of data and visualizing results, (2) the ability to store and retrieve large amounts of data, and (3) opportunities for testing mathematical models based on experimental results. This project most closely supports Objective 4, Approach 4 of the "National Cancer Plan" by providing methods of developing mathematical models of the cancerous process.

Proposed Course of Study: Programs will continue to be developed and computational techniques to be applied in response to computer-related needs of the scientific staff.

Serial No. NCI-[illegible]
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Analysis of Mechanisms Underlying Determination and Transdetermination In Drosophila

Previous Serial Number: None

Principal Investigator: Stuart Kauffman

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: Determination and transdetermination in imaginal disks of Drosophila must be underlain by some form of control system having sharply alternate self maintained states. If many such subsystems or circuits are involved, the combinations of their states may be characterized as an epigenetic code. The major objective is to uncover the logical structure of that code. Recently, I suggested determination is due to at least four circuits each with two alternate states. Thus leg ≡ 1010, antenna ≡ 1100, wing ≡ 1111, where 1 is the more stable state of each circuit. To test this model, I hope to achieve somatic cell fusion of disk cells, then test the determined state of the fused cell. If the more stable "1" state wins any competition, leg x wing = 1010 x 1111 yields 1111 or wing, while haltere fused with antenna (0111 x 1100 → 1111) should yield wing.

Methods Employed: Dr. H. Schneiderman has supplied three complementing temperature sensitive cell lethal mutants of Drosophila. Each carries a second visible cuticle marker. I am disaggregating and coreaggregating 3rd instar imaginal disks of two T.S. strains, treating with various chemical and viral agents to induce fusion, implanting the aggregate in 3rd instar larvae to induce the metamorphosis of the reaggregate with the host, and hoping to find fused cells. Such cells should be characterized by being wild type for cuticle markers, and wild type for the complementing T.S. mutants, hence I should be able to select for rare fusion events at the nonpermissive temperature and recognize fused cells.

Major Findings: None. The techniques are difficult and barely in hand.

Serial No. NCI-8313
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance for Cancer Research: Imagine that one knew for each pair to be fused (e.g., wing x leg → ?) what it yielded. The matrix listing all possible pairs and the result will be a powerful clue to the logical structure of the decision processes underlying determination and transdetermination. I take it that illuminating that structure and its material make up constitute the central problem of developmental genetics. Objective 3, Approach 1 of the National Cancer Plan applies.

Proposed Course: Try various agents, lysolecithin, concanavalin A, sendai virus, in attempts to obtain fusion.

Serial No. NCI-8314
1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Modeling of the Adenylate-Cyclase System

Previous Serial Number: None

Principal Investigators: Marc Rendell and Mones Berman

Other Investigators: Martin Rodbell (NIAMDD)

Cooperating Units: Section of Membrane Regulation
Laboratory of Nutrition and Endocrinology, NIAMDD

Man Years:

Total:	2.5
Professional:	2.5
Others:	0

Project Description:

Objectives: The kinetics of adenyl cyclase is being investigated in the fat cell, hepatocyte, and the adrenal. Present studies have concentrated on the catalytic site and the nucleotide regulatory site of the enzyme. In the future, it is anticipated that the hormone receptor will be integrated into the picture to give a complete kinetic description of the adenyl cyclase unit in these cells.

Methods Employed: Sophisticated techniques of simulation and analysis are being used for the first time in connection with enzyme kinetic experimentation on a pharmacologic system. These techniques are used not only for classic data fitting but also to determine what information is needed to define the system.

Major Findings: The effect of magnesium on cyclase has now been resolved as due to its effect of reducing the concentration of HATP in the system. The K_m for MgATP and the K_I for HATP have been determined. Hepatic cyclase has been resolved into two fundamental states with different K_m, K_I, and V_{max}. A model has been proposed for the nucleotide regulatory site.

Significance to Biomedical Research and the Program of the Institute: This work comes under the National Cancer Plan 4,4. It is known that transformed cells have different magnesium responses than normal cells. This work could bear directly upon this phenomenon. Future course will be to extend this work to other cells and to define the hormonal factor.

Summary Outline

Introduction

I. Structure and Function of Immune Cell and Neoplastic Cell Membranes and Cell Products

- A. Membrane Receptors
- B. Normal Cell Surface Antigens
- C. Tumor Cell Surface Antigens
- D. Modified Cell Surfaces
- E. Cell Products

II. Cellular Immunity

- A. Separation and Characterization of Lymphoid Cell Populations
- B. Antibody Dependent Lymphocyte Mediated Cytotoxicity

III. Genetics of the Immune Response

- A. Genetic Control of Responses to Staphylococcal Nuclease
- B. Genetic Control of Responses Not Linked to H-2
- C. Genetic Control of Responses to Altered Cell Surface Antigens
- D. Relationship Between Ir Genes and Immunoglobulin Variable Region Genes

IV. Transplantation Immunobiology

- A. Antigens of the Major Histocompatibility Locus
- B. Transplantation Antigens of Swine
- C. Pre-clinical Model of Bone Marrow Transplantation
- D. HL-A and Disease Associations

V. Structure and Immunochemistry of Immunoglobulins

- A. Three Dimensional Structure of Immunoglobulins
- B. Idiotypic Studies of Human Myeloma IgA

VI. Immunodiagnosis

- A. Carcinofetal Proteins

VII. Clinical Immunotherapy

- A. Melanoma
- B. Glioblastoma
- C. Skin Cancers
- D. Leukemia

SUMMARY

Annual Report of the Immunology Branch, National Cancer Institute
July 1, 1973 through June 30, 1974

INTRODUCTION

The Immunology Branch carries out basic immunobiology laboratory investigations with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation. The fundamental information obtained is then applied to investigations of the interactions between the immune system and neoplastic cells, as well as to the possible diagnosis and therapy of malignant disease in animal models and in man.

I. STRUCTURE AND FUNCTION OF IMMUNE CELL AND NEOPLASTIC CELL MEMBRANES AND CELL PRODUCTS

A. Membrane Receptors. The function of cells, including cells of the immune system, is partially initiated and modulated by interactions between cell surface receptors and a variety of ligands. One such receptor on the B cell subpopulation of human lymphocytes is a receptor for antigen-antibody complexes or aggregated IgG (5035). This receptor has been partially characterized and appears to play a role in the interaction between non-immune B cells and antibody coated target cells that leads to cell lysis (see II., B.).

Mitosis of lymphoid cells is initiated by interactions between "mitogens" and cell surface receptors. Studies indicate that PHA and Con A bind to similar, or possibly identical, membrane receptors (5018).

Human lymphoid cell lines that have cell surface immunoglobulin (B-cell lines) were shown to have receptors for insulin and growth hormone. T-cell lines, cell lines derived from Burkitt lymphoma and Burkitt lymphoma cells obtained by biopsy appear to lack these receptors. These receptors have been further characterized (5004). Studies of mouse lymphoid cells and human peripheral blood lymphocytes indicate that only populations of cells containing phagocytes seem to have insulin receptors (5039). The apparent discrepancy between the results of 5004 and 5039 may indicate that lymphoblastoid B-cell lines in long term culture express receptors not present on normal B-cells. Lymphoid cell receptors for glucocorticosteroids have also been studied (5039).

Cell surface IgD on human lymphoid cell lines and IgE receptor concentration on rat basophils have also been studied (5004).

B. Normal Cell Surface Antigens. Human HL-A antigens have been further characterized (5006). A system was devised which permits solubilization of immunochemically recognizable intact HL-A molecules and they have been compared with papain fragments of HL-A molecules.

Human lymphoid cells have been further characterized in terms of cell surface antigens with the use of antisera specific for T-cell populations, and other antisera specific for B-cell populations. Use of these antisera has permitted analysis of T and B cell populations in patients with a variety of diseases (5031).

C. Tumor Cell Antigens. Lymphoid cells from patients with acute leukemia contain a cell surface antigen not found on other lymphoid cells. Antisera have been prepared against this antigen and have been used to immunochemically separate the solubilized antigen from HL-A3 (5006).

Antisera have been prepared against cells of patients with chronic lymphocytic leukemia, and absorbed with parental cells to remove antibodies against inherited cell surface antigens. These antisera appear to detect an antigen specific for CLL cells (5024).

Antisera have been prepared against cultured melanoma cell lines and absorbed with cultured human lymphoblast cell lines. These antisera demonstrate an antigen shared in common between melanoma cell lines, explanted melanoma cells and nevus cells. The antigen was not detectable on breast or ovarian cancer cells or skin fibroblasts (5029).

D. Modified Cell Surface. Lymphoid cells have been conjugated with a number of haptens and it has been shown that these modified cells are immunogenic in syngeneic animals (5003), as well as in allogeneic animals (5037, 5038). Modified cells are a model system for studying effector cell activity against well defined cell surface antigens.

Treatment of cell surfaces with the enzyme neuraminidase alters them in such a way that they react with a "natural" antibody present in normal human serum. The chemical nature of this antigenic determinant has been studied with hapten inhibition experiments and it has been shown that carbohydrates containing β-D-galactopyranosyl residues specifically inhibit the reaction between antibody and modified cell surfaces (5017).

E. Cell Products. Carcinoembryonic antigen is secreted by certain malignant cells. Amino acid sequence studies of the protein portion of this molecule have been carried out, and proof has been obtained that the molecule being sequenced is the same molecule detected in the radioimmunoassay for CEA (5019).

Human lymphoid cell lines produce a factor which activates osteoclast activity (5004). This may be the factor responsible for osteolysis in multiple myeloma.

II. CELLULAR IMMUNITY

A. Separation and Characterization of Lymphoid Cell Populations. The use of nylon wool for separation of lymphoid cells has been modified so that adherent cells can be recovered (5039). Separated cell populations have been functionally characterized. Adherent cells (B-cells) appear to be better stimulators of MLC interactions, and non-adherent cells (T-cells) appear to be better responders. Separated cell populations have also been studied in allogeneic in vitro immunization experiments and have indicated that there is synergy between adherent and non-adherent responders (5039).

Human peripheral blood lymphocytes consist of 4 subpopulations: B cells, T cells, cells bearing both B and T cell markers, and cells devoid of markers (5034). Expression of cell surface markers correlate with clinical designations of variable immunodeficiency and panhypogammaglobulinemia (5034).

Murine lymphoid cells have been separated on sepharose columns coated with histamine. Cells of different functional characteristics can be separated by this procedure, thus providing another technique for studying lymphoid cell function (5028).

Recent experiments have suggested that some lymphoid cells have a regulatory function relative to other lymphoid cells. Apparent suppressor cell function has been demonstrated by showing that removal of adherent cells (B-cells) from the spleen of tumor bearing mice increased the degree to which non-adherent cells could be sensitized against allogeneic tissue (5003). Adherent cells depress the degree to which non-adherent spleen cells from normal mice can be sensitized against allogeneic tissue. Tumor bearing mice, therefore, seem to produce adherent cells, presumably B-cells, which can non-specifically suppress cytotoxic immune responses.

Experiments have been performed which can be interpreted as indicating that cytotoxic T cells are a different population than the T cells that express memory for a cytotoxic response (5003).

B. Antibody Dependent Lymphocyte Mediated Cytotoxicity. Studies on various components of this system have been carried out. The class of antibody required to sensitize target cells appears to be IgG, but it is possible that only some of the subclasses may mediate this effect (5006).

The effector cell in this system appears to be a B cell, at least when nucleated target cells are used (5016). Effector cell activity can be inhibited by exposing effector cells to aggregated IgG. It seems possible, therefore, that the receptor site for aggregated IgG (Fc receptor) is the same receptor required for interaction between attacker and antibody-coated target.

Treatment of attacking lymphocytes with trypsin under conditions where the Fc receptor is unaffected caused decreased killing activity. This suggests that antibody dependent lymphocyte mediated cytotoxicity is at least a 2 stage process, involving the Fc receptor and other as yet undefined cell surface components (5018).

A model system has been established in which TNP coated red cells are used as targets. This provides a well defined antigen. Anti-TNP IgG sensitizes these cells for lysis by appropriate attacking cells. Neither IgM nor IgA will sensitize, and an IgA myeloma protein can be shown to competitively block sensitization by IgG (5018).

III. GENETICS OF THE IMMUNE RESPONSE

A. Genetic Control of Response to Staphylococcal Nuclease. In studying the genetic control of immune responses, it is desirable to utilize antigens of known structure. Staph nuclease meets this requirement. H-2 linked genetic control has been demonstrated and localized to the Ir-1 region. Significant differences between the number of cells synthesizing IgM antibody in high and low responder animals were detected, a result different from that seen with synthetic polypeptide antigens. Antibodies produced by high and low responder strains appear to be specific for an antigenic determinant located in the N-terminal 126 residues of the nuclease molecule (5025).

Much work on genetic control has been concerned with antibody synthesis. Using staph nuclease it has been possible to show that this antigen stimulated protein synthesis in cells of high responder, but not in cells of low responder strains. This assay correlates with cell mediated immunity, thus indicating that genetic control in this system is also exerted over cell mediated immune responses (5026).

B. Genetic Control of Immune Responses Not Linked to H-2. Analysis of antibody responses to the loop peptide of lysozyme, as well as to the synthetic polypeptide Pro--L indicates that the genes controlling these responses are not H-2 linked. This demonstrates an additional complexity in the genetic control of immune responses (5027).

C. Genetic Control of Immune Responses to Altered Cell Surface Antigens. TNP-modified spleen cells are immunogenic in syngeneic animals and lead to a cellular immune response directed against a cell surface antigen which may itself be under genetic control and may represent an Ir gene product. Ability to respond to TNP-modified cells is H-2 linked and is dominant. This work is in its preliminary stages but may provide an indirect method for identifying Ir-gene products (5038). Modification with carboxy pyridine disulfide (CPDS) also permits identification of an H-2 linked system of control of responsiveness to modified syngeneic lymphocytes (5037).

D. Relationship Between Ir Genes and Immunoglobulin Variable Region Genes. Studies are being carried out to determine whether Ir genes control the immunoglobulin variable regions that are produced in "genetically controlled" antibody responses. Experiments have progressed to the point where specifically purified mouse anti-nuclease antibodies have been prepared and used to immunize rats. Rats have made antibodies against the idiotypic determinants of the mouse antibody. Rat antisera will be used to test linkage between H-2 and specific idiotypes (5036).

IV. TRANSPLANTATION IMMUNOBIOLOGY

A. Antigens of the Major Histocompatibility Locus. Antisera have been prepared which detect a new series of antigens that are controlled by genes located close to the Ir genetic region. These immune response associated antigens have been designated Ia antigens and have been shown to play a role in stimulating mixed leukocyte interactions. The antigens appear to be expressed preferentially on B cells and are thus another B-cell differentiation marker (5021).

B. Transplantation Antigens of Swine. A breeding program has been initiated with miniature swine. Parents and offspring are histocompatibility antigen typed and results are used for further mating. This should provide a rapid method for developing animals homozygous at the major histocompatibility locus (5023).

C. Pre-clinical Model of Bone Marrow Transplantation. Rhesus monkeys are being used to study allogeneic bone marrow transplantation and graft versus host disease. New histocompatibility antigens have been discovered. ALS and irradiation schedules are being tested in an attempt to maximize graft acceptance and minimize GVH (5011).

D. HL-A and Disease Associations. Patients with various diseases are HL-A typed and associations sought between specific antigens and disease. For example, the normal frequency of HL-A8 is 22%. By contrast, 60% of patients with dermatitis herpetiformis and 90% of patients with gluten sensitive enteropathy carry the HL-A8 antigen. The biologic significance of histocompatibility antigens is not yet known. Identification of diseases associated with abnormal frequencies of HL-A antigens may provide clues to the functions of this system (5017).

V. STRUCTURE AND IMMUNOCHEMISTRY OF IMMUNOGLOBULINS

A. Three Dimensional Structure of Immunoglobulins. A mouse myeloma protein that binds phosphorylcholine has been crystalized and the structure determined at 3.1 A^{o} resolution. The hapten binding site has been located in a cleft formed by the heavy chain hypervariable regions (5040).

B. Idiotypic Studies of Human Myeloma IgA. The myeloma protein of a young patient with myeloma has been isolated, and anti-idiotype antisera prepared. The anti-idiotype has been used to monitor quantitative levels of myeloma protein in the patients circulation, as well as to monitor cells secreting that idiotype in the patients bone marrow. This approach may provide a means for closely monitoring therapy in patients with myeloma (5022), and might ultimately have therapeutic implications in myeloma.

VI. IMMUNODIAGNOSIS

A. Carcinofetal Proteins. A laboratory for determining CEA concentration has been established by contract. CEA is determined with a double antibody assay, and the facility is being used in support of cooperative group trials, as well as a number of trials at NCI. A cooperative study to compare the CEA concentrations obtained with different assays was organized. All assays provided comparable results, except for the hemagglutination-inhibition assay which did not seem to detect CEA.

VII. CLINICAL IMMUNOTHERAPY

A. Melanoma. Eighteen stage III and 9 stage II patients have been entered into a study of the effectiveness of immunization with neuraminidase treated, cultured, allogeneic cells, given with BCG. The study has not run long enough to provide interpretable results (5033 and 5016).

B. Glioblastoma. Patients who have had glioblastomas removed are immunized with BCG and treated intracranially with PPD. This study is too early to evaluate (5033).

C. Skin Cancers. The first phase of a study of the effectiveness of DNCB on basal cell cancers has been completed. It was found that some, but not all, lesions can be cured with DNCB applications in a pre-sensitized individual. Additional skin cancers will be studied (5033).

D. Leukemia. Patients with acute leukemia who have relapsed a number of times are brought into remission with chemotherapy, and are immunized with the Raji cell line. Patients have made immune responses to the cell line, but no evidence of therapeutic effectiveness has been noted as yet (5006).

Serial No. NCI-5003(c)
1. Immunology Branch
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cellular Immunity I

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Arnold Eggers, M.D. ; Hillel Koren, Ph.D.;
Elizabeth Simpslon, D.V.M.

Cooperating Units: Janet Hartley Ph.D., and John Inman, Ph.D.

Man Years:

Total: 5
Professional: 3
Other: 2

Project Description:

Objectives: This project has been directed at understanding and manipulating mechanisms of cell-mediated immune cytotoxicity. Long range goals are: (1) to identify and characterize factors which influence induction, maturation, and expression of a cellular immune response (2) to define changes in cellular immunity which occur in tumor bearing hosts; (3) to find means of manipulating the cellular immune response for therapeutic purposes. Immediate objectives of the past year have been to concentrate on goals #2 and 3.

Methods Employed: Direct, cell-mediated cytotoxicity has been measured in vitro by release of ^{51}Cr from labelled target cells incubated with immune lymphoid cells for 2-4 hours. Immune cells are collected from spleen, marrow, lymph node, thymus and peripheral blood of sensitized hosts. In one series of experiments, spleen cells were fractionated on nylon columns by the method of Julius et al. (Eur. J. Immunol., in press). The procedure removes most monocytes, macrophages and B lymphocytes. Effluent cells are highly enriched for T lymphocytes. In addition, immune cells have been produced by in vitro sensitization of spleen cells from normal donors using previously established Mishell and Dutton tissue culture techniques. Target cells are provided by freshly explanted tumors, tumor tissue culture lines, and normal lymphoid lines. Target cells have also been chemically modified not only for use as target cells in ^{51}Cr release assays but also for use as in vitro immunogens.

Chemical modification has been with TNP sulfonate and also with NIP, dansyl and arsonyl conjugates. All reactants are particularly advantageous in that they combine covalently with cell surfaces under physiologically mild conditions.

Major Findings: Relationship of effector cell and memory cell activity for cell-mediated cytotoxicity. Little is known about the relationship of cytotoxic T lymphocytes and the memory cells from which they are derived. During the latter part of her sabbatical year in the Immunology Branch, Dr. Elizabeth Simpson investigated this question by testing mice sensitized with allogeneic skin grafts. She used the 4-hr ^{51}Cr release cytotoxicity assay as a test for cytotoxic lymphocytes and a 5-day Mishell-Dutton in vitro immunization procedure followed by the cytotoxicity assay as a test for memory cells. Memory cells were defined as those cells capable of giving a secondary type cytotoxic T-cell response following re-exposure to antigen. She found that cytotoxic activity peaked and disappeared within 3 weeks after sensitization. Memory activity, however, persisted for at least 14 weeks after sensitization. Both cytotoxic and memory activity were T-cell dependent and were antigen specific. These results sustain the view that cytotoxic T cells and memory T cells for the cytotoxic response occur as two separate cell populations.

Augmentation of Cytotoxic Cellular Immunity in Lymphoid Cells from Tumor Bearing Hosts by in vitro Sensitization of Fractionated Host Lymphoid Cells. Dr. Arnold Eggers has tested the ability of lymphoid cells from tumor bearing hosts to develop cytotoxic cellular immune responses in vitro to antigens unrelated to the tumor. He has used the 4-hr ^{51}Cr release cytotoxicity assay and Mishell-Dutton procedures for sensitization in vitro. These procedures have served as tests for effector stage immune activity and host cellular immune responsiveness. He found that spleens of C57BL/6 mice bearing a transplanted MCA-induced C57BL/6 sarcoma developed poor cellular immune responses in vitro to C3H/HeN transplantation alloantigens. The cellular immune response, which was compared to that of normal donor spleen cells was increased to normal levels if the responder cells were fractionated on nylon columns and only non-adherent cells were sensitized. The nonadherent cells were primarily T lymphocytes. The nylon adherent cells from tumor-bearing donors suppressed the response of T lymphocytes from either normal donors or tumor bearing donors. The "suppressor" cells were not sensitive to anti-T cell reagents but they do appear to be sensitive to anti-mouse B lymphocyte reagents (anti kappa plus complement). Although present evidence argues that suppressor cells are B lymphocytes, plams from tumor bearing hosts and supernatants from tumor host spleen cells have failed to suppress cellular immune responses.

These findings argue that tumor bearing hosts can produce B lymphocytes which non-specifically suppress the cytotoxic immune response. If the suppressor cells are removed by procedures such as nylon column filtration, the remaining lymphoid cells from tumor bearing hosts function normally in developing cytotoxic immune cell activity.

Immunogenicity of Chemically Modified Mouse Cells. Dr. Hillel Koren is exploring the effects of a variety of chemicals on cell immunogenicity. These compounds, which covalently combine with cell surface lysyl epsilon amino groups through reactive acyl azides, have been provided by Dr. John Inman. These compounds share the properties of water solubility and non-toxic reactivity with viable cells under physiologic conditions. In addition, they share the same reactive side group and consequently should bind to the same cell surface components. Initial efforts have tested the possibility that these compounds will render normal cells immunogenic. Results have demonstrated that C57BL/6 spleen cells from normal donors indeed respond in vitro to syngeneic spleen cells modified by dansyl, arsanyl, or NIP conjugates. Sensitized spleen cells are cytotoxic but demonstrate specificity in that they damage only those target cells modified with the haptene used for sensitization. Thus, C57BL/6 cells sensitized against syngeneic NIP-modified cells damaged NIP modified syngeneic targets but not unmodified or Ars-modified syngeneic targets. Anti T serum plus complement obliterated cytotoxic activity, arguing that the killer cells are T. cells.

Stimulation of Anti-tumor Immunity by Non-specific Antigen Sensitization or by host Lymphoid Cell Fractionations. John Ryan, a graduate student and technician in the laboratory, is working on responsiveness of AKR mice to AKR leukemias as a thesis project. He has used the 4-hr ^{51}Cr-release test to measure cell-mediated cytotoxic activity against spontaneous AKR leukemia which we adapted to in vivo passage. He has found that 1) unfractionated spleen cells from AKR mice are not cytotoxic for AKR leukemia cells; 2) AKR spleen cells which fail to adhere to nylon columns are cytotoxic for the leukemia cells; 3) in vitro sensitization of normal AKR spleen cells with AKR leukemia does not induce cytotoxic anti-leukemia activity; 4) in vitro sensitization of AKR spleen cells by normal allogeneic spleen cells induces cytotoxic activity against the leukemia cells; and 5) in vitro sensitization of AKR spleen cells previously sensitized in vivo with sheep red blood cells induces cytotoxic activity against the leukemia cells. We tentatively interpret these preliminary but important findings as follows:

Non-tumor bearing AKR mice have cytotoxic cells capable of destroying leukemia cells. Why it is necessary to remove nylon adherent cells from the attacking cell population in order to observe activity is uncertain. Stimulation of host cells by antigens unrelated to leukemia cells stimulates anti-leukemia activity, perhaps by a Katz-Benacerraf type of allogeneic effect.

Establishment of an Operational Cell Sorter. In December of 1973, the Immunology Branch obtained a copy of the Stanford Herzenberg Fluorescent Activated Cell Sorter. The machine scans individual cells with a laser beam and sorts them at the rate of about 1,000 cells per second. Cells are analyzed for light scatter, a reflection of cell size, and intensity of fluorescence. Cells have been pre-labelled with a fluorescent marker. The Sorter will be available to the Immunology Branch and to the scientific community. It will be supported by one full-time technician from the Immunology Branch.

Significance to Biomedical Research and the Program of the Institute: (1) Relationship of effector cell and memory cell activity in cell mediated cytotoxicity. In our previous and current investigations of cell mediated immunity we have relied heavily upon a brief ^{51}Cr-release cytotoxicity assay. This type of assay reveals pulses of cellular immune activity following host sensitization with syngeneic tumor or allogeneic tissues. Immune activity peaks at about two weeks and disappears by 3 weeks. In vivo relevance of this assay to cellular immunity in vivo has been querried on the basis of positive delayed hypersensitivity skin tests or second set skin graft rejections persisting for years after primary sensitization. Our interpretation of these phenomena is that positive tests for cellular immunity which require several days for completion, whether in vivo or in vitro, can reflect memory or precursor cell activity in addition to or instead of cytotoxic effector cell activity existing at the time of antigenic challenge. This interpretation is supported by our finding that memory for allogeneic skin grafts persists for at least 14 weeks following primary sensitization, whereas cytotoxic cell activity lasts for only 3 to 4 weeks. This finding is important for understanding the relationship of our in vitro cytotoxicity tests to in vivo phenomena and for our interpretation of other longer term in vitro tests for cell mediated cytotoxicity. Many in vitro tests utilize 2 to 3 day incubation periods which may permit in vitro conversion of memory cells to cytotoxic cells and consequent detection of memory cell acitivity.

(2) Augmentation of cytotoxic cellular immunity by host lymphoid cell fractionation. In the particular mouse model system used for these studies, non-specific suppression of cytotoxic cellular immune responsiveness resulted from a subpopulation of lymphoid cells which could be removed by nylon column fractionation. the identification of such a suppressor cell subpopulation offers the possibility that the mechanism of this type of suppression can be elucidated. At present, a normal immune response can be established in vitro, and this step may prove valuable for passive immunotherapy.

(3)Chemical Modification of Mouse Cell Surface Antigens. A particularly promising aspect of this work is that is offers a model system for studying specificity of cytotoxic T-cells for well-defined minor cell surface antigens. In addition, use of this system offers the possibility of chemically modifying tumor cell surfaces, rendering tumor-associated antigens more immunogenic.

(4) Stimulation of Mouse Anti-tumor Immunity by Non-specific Antigen Sensitization and by Host Lymphoid Cell Fractionation. In addition to chemical modification of tumor cells, host anti-tumor responsiveness has been stimulated by immunization with sheep red blood cells and by separation of host lymphoid cells by nylon columns. These findings are important because they argue that tumors are immunogenic, that host responses are suboptimal. They also argue that at least several and perhaps many operationally independent methods exist for improving host-anti-tumor immune responses. If one method should fail in an immunotherapy setting, perhaps another method or combination of methods for improving host responsiveness will succeed. The explanation for suboptimal host responsiveness is only fragmentary and clearly requires further investigation.

([illegible]) Establishment of an operational Cell Sorter. This machine, which is a copy of the Stanford Fluorescent Activated Cell Sorter, scans individual cells in a fluid stream at about 1,000 per second. The cells are sorted on the basis of fluorescent intensity or size, and recovered cells are both viable and biologically functional. It is anticipated that the Sorter will provide a service to the Immunology Branch and the general scientific community. We expect that the Sorter will greatly aid categorization of host cells participating in an anti-tumor immune response and identification of tumor cell properties which will render these cells particularly immunogenic or susceptible to immune destruction.

Honors and Awards: None

Publications:

Martin, W. J., Wunderlich, J. R. and Macdonald, J.: Suppressed development of cytotoxic lymphoid cells in tumor-immunized mice. Israel J. Med Sci 9: 324-331, 1973.

Wunderlich, J. R., Martin, W. J. and Macdonald, J.: Functional efficiency of antitumor cytotoxic lymphoid cells. Israel J. Med. Sci. 9: 317-323, 1973.

Yust, I., Wunderlich, J.R., Mann, D. L., Rogentine, G. N. Jr., Leventhal, B., Yankee, R. and Graw, R.: Human lymphocyte dependent antibody mediated cytotoxicity against non-HL-A antigens. Nature, in press.

Yust, I., Wunderlich, J. R., Mann, D. L. and Terry, W. D.: Identification of lymphocyte dependent antibody in sera from multiply transfused patients. Transplantation, in press.

Serial No. NCI-5004(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Regulation of Growth and Biosynthetic Activity of Cells of the Immune System

Previous Serial Number: Same

Principal Investigator: Donald N. Buell, M.D.

Other Investigators: None

Cooperating Units: John Van Boxel, M.D., Arthritis and Rheumatism Branch, NIAMDD; Henry Metzger, M.D., Arthritis and Rheumatism Branch, NIAMDD; Chavina Isersky, M.D., Arthritis and Rheumatism Branch, NIAMDD; Jesse Roth, M.D., Clinical Endocrinology Branch, NIAMDD; Steven Marx, M.D., Clinical Endocrinology Branch, NIAMDD; G. Mundy, M.D., University of Rochester School of Medicine; L. Rais , M.D., University of Rochester School of Medicine

Man Years:

Total:	1
Professional:	1
Other:	

Project Description:

Objectives: 1) The development of large scale, long-term cultures of functionally capable immune cells. 2) Delineating the factors which play a role in control of growth and proliferation of cells of the immune system. 3) Understanding the mechanism which regulates lymphocyte differentiation.

Methods Employed: Tissue culture of human lymphoid cells is an important part of this work. Immunofluorescent cytology and radioimmunoelectrophoresis are used to identify specific immunoglobulins produced or present on the cell surface. Rates of production and turnover are studied by continuous and pulse label incubations with radioactive amino acids, with analysis of incubation medium and cytoplasm using solid phase radioimmunoassay. Standard counting techniques are used to follow cell growth. Incorporation of specific labeled precursors followed by autoradiography and scintillation counting are used to study rates of RNA and DNA synthesis, and to determine cell cycle parameters. Synchrony of cell growth is achieved by double thymidine and thymidine-colcemid blockade. Rate zonal centrifugation is used to separate cells according to size, cell cycle position and functional properties. Special culture techniques are used to establish high density stationary phase cultures, which on resumption of exponential growth synchronously enter the G_1 phase of the cell cycle.

Freeze-thaw elution and detergent solubilization permit isolation of plasma membranes. Lactoperoxidase radioiodination is used to label cell surface proteins in intact cells. Proteins for cell receptor binding studies are iodinated by the chloramine T method. Specific cell surface receptors are identified by measuring the binding of radiolabelled ligands. Altered cell growth conditions, temperature shifts, enzyme treatment and additions of specific drugs are used to investigate the turnover of membrane components. An IgE binding rat basophilic leukemia is maintained as a cell line in suspension culture. IgE receptors are quantitated by saturation binding with ^{125}I-labelled IgE purified from rat myeloma serum.

Major Findings: A large number of human lymphoid B cell lines were examined and found to vary significantly in the quantity of surface insulin and growth hormone receptors. Cell lines derived from Burkitt lymphoma biopsies were found to have striking deficits of these receptors compared to other human lymphoid lines studied. A number of Burkitt lymphoma biopsies have been examined and show hormone receptor deficits similar to the BL derived cell lines. Three human T cell lines have been examined. All three lack insulin and growth hormone receptors.

Attempts have been made to identify receptors for additional hormones in human lymphoid cell lines. Calcitonin binds to receptors on both B and T cell lines. As yet no receptors for human chorionic gonadotropin (HCG), human placental lactogen (HPL) or thymopoietin (thymin) have been found in the cell lines examined.

Modulation of specific hormone receptors has been more fully characterized in human lymphoid cell lines. Hormone receptor concentration is dependent upon the concentration of hormone to which the cells are exposed. Growth in insulin at 10^{-8}M, a concentration which occupies 75% of binding sites, will cause a steady state loss of 50% of membrane insulin receptors. The corresponding mechanism for growth hormone is more sensitive with 10% occupancy of sites resulting in a 50% decrease in the membrane hormone receptor concentration. Hormone dependent regulation of hormone receptor concentration has been found to be hormone specific and to have time and temperature dependence which are clearly different from simple binding.

Surface IgD has been found on a relatively large proportion (6/25) human lymphoid cell lines examined. When found, IgD has been associated with multiple other surface immunoglobulins - IgG, IgM, IgA but not IgE. Each of these classes of surface immunoglobulin has been shown to migrate independently in the membrane (independent capping).

IgE receptor concentration in rat basophils varies with both the state of growth of the cell culture and as a function of the phase of the cell cycle.

A threefold increase in IgE receptors is seen after cell growth ceases in high density stationary phase. Synchronization studies indicate that IgE receptors appear in the membrane during the G_1 phase of the cell cycle. Rapidly growing basophils are relatively undifferentiated, resembling promyeloblasts. Growth arrest in high density stationary phase occurs in the G_1 part of the cell cycle. Striking differentiation is seen as measured by basophilic granule maturation, histamine content and IgE receptor concentration.

Human lymphoid cell lines derived from multiple myeloma, Burkitt and non-Burkitt lymphomas produce an osteoclast activation factor which promotes bone resorption. This has been characterized and found to differ from other substances known to promote osteoclastic bone resorption.

Significance to Biomedical Research and the Program of the Institute: 1) Key questions in immunology and cell biology in general center upon the nature of the alterations in molecular activity of a cell attendant upon binding of a specific activator molecule to its cell surface membrane. The finding that some but not all lymphocytes possess insulin receptors may open up new directions of investigation in these areas. 2) It is clear that much of the regulation of activity of mammalian cells is mediated by binding of specific effector molecules by cell surface receptors. Loss of critical cell surface receptors, analogous to the deficits of insulin and growth hormone receptors in Burkitt lymphoma and derived cell lines, may be an explanation of neoplastic cell beh-vior. 3) A feature of cellular differentiation is the appearance of specific receptors in the cell membrane. Thus cells undergoing functional maturation also become susceptible to specific regulatory molecul . 4) Hormone specific receptor modulation in lymphoid cell lines appears to be a model for a very important biological regulatory mechanism. Cells are protected from overstimulation by effector molecules by active reduction of their corresponding membrane receptors. 5) IgD is found with high frequency as a surface immunoglobulin in normal and neoplastic B lymphocytes and is speculated to have an important biological function. The existance of cell lines bearing IgE and multiple other surface immunoglobulins provides a powerful tool for investigation. 6) The production of osteoclast activating factor(s) probably represents the causative mechanism of destructive bone lesions seen in multiple myeloma, Burkitt lymphoma and other lymphoid malignancies.

Honors and Awards: None

Publications:

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Mundy, G. R., Luben, R. A., Raisz, L. G., Oppenheim, J. J., and Buell, D. N.: Bone resorbing activity in supernates from lymphoid cell lines. N. Eng. J. Med. 290:867-871, 1974.

Serial No. NCI-5006(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: I) Transplantation Antigens
II) Acute Leukemia Associated Antigens
III) Characterization of Lymphocyte Dependent Antibody

Previous Serial Number: Same

Principal Investigator: Dean L. Mann, M.D.

Other Investigators: Israel Yust, M.D., June Miller, Ph.D., John Wunderlich M.D.

Cooperating Units: Brigid G. Leventhal, M.D., Medicine Branch, NCI

Man Years:

Total:	3
Professional:	3
Other:	0

Project Description:

Objectives: I - Transplantation Antigens: The objectives of the studies are fourfold: a) to purify human transplantation antigens; b) to chemically, genetically and immunologically characterize these antigens; c) to use soluble HL-A alloantigens to raise allotypic antisera in heterologous species; d) to define and characterize cell surface components that function as antigen receptor sites and relate them to HL-A alloantigens. This work supports Objective No. 3, Approach No. 5. II - Acute Leukemia Associated Antigens: a) Identification of an antigen associated with acute leukemia. b) Isolation and characterization of this antigen; c) use antigen to produce heterologous antiserums; d) identify factor(s) in serum of leukemic patients that inhibit and/or promote cell-mediated cytolysis, to infect non-human cell lines with murine leukemia viruses and identify new antigens that cross react with the human acute leukemia antigen. In conjunction with this study and based on previous observation, an immunotherapy program was initiated with Dr. Leventhal of the Medicine Branch, NCI. Patients with acute leukemia were injected with RAJI cells. This laboratory has been undertaking the assessment of the immunologic response of these patients to this immunization procedure. This work supports Objective 6, Approach 3. III - Characterization of Lymphocyte Dependent Antibody: A serum component (antibody) mediating cellular cytotoxicity (LDA) will be identified and characterized. Patients with autoimmune disease and malignancies will be screened for the presence of this antibody. This work supports Objective No. 6, Approach 4.

Methods Employed: ^{51}Cr release and dye exclusion cytotoxicity tests (See Project Report NCI-5008) were used to detect alloantigens. Human cells were obtained from normal blood bank donors and from long term tissue culture. Antisera detecting genetically determined human leukocyte alloantigens were obtained as described in Project NCI-5008. Alloantigens in subcellular form were detected by a cytotoxic inhibition assay using the ^{51}Cr release from target cells and employing the human leukocyte typing antiserums.

Human leukocyte alloantigens were prepared by graded hypotonic lysis of cell suspensions. Membrane fragments were treated with papain and detergents to yield a water soluble antigen. Further purification was done by CM-Sephadex chromatography, gel filtration chromatography, electrofocusing and acrylamide gel electrophoresis. The physical and chemical properties of the antigens were investigated.

To aid in studies of identification and isolation of HL-A alloantigens, heterologous antiserums were raised by injecting purified HL-A alloantigens into rabbits. These antiserums were evaluated for cytotoxicity to human lymphoid cells as well as for their ability to precipitate solubilized HL-A alloantigens. The antisera was absorbed with cells in attempts to raise specific allotypic antisera. The precipitation techniques were utilized to isolated HL-A bearing alloantigens.

The acute leukemia associated antigen(s) was detected in dye exclusion and ^{51}Cr chromium release cytotoxicity assays. Antiserum used in these studies were obtained by immunizing rabbits with water soluble cell membrane preparations from the tissue culture cell line RAJI as well as serum from patients immunized with RAJI cells. Peripheral blood lymphocytes from patients with acute leukemia were labeled with ^{51}Cr, incubated with autologous serums or serum from other leukemic patients and/or with heterologous antiserum that detect the acute leukemia antigen. Normal peripheral blood lymphocytes were added and the lysis or inhibition of lysis mediated by the various serums determined. Patients injected with the RAJI cell lines were assessed for the development of LDA and complement dependent cytotoxic antibody. Serum from these patients were screened for substances that blocked cytotoxic reactions.

Serums were obtained from patients who had received multiple transfusions or had a diagnosis of autoimmune disease or malignancies. These serums were tested in the antibody mediated cellular cytotoxicity reaction using various target cells and normal peripheral blood lymphocytes. The serums tested from the various patients were screened for their ability to mediate the cellular cytotoxicity reaction (LDA). The positive sera were fractionated by gel filtration, ion exchange chromatography, and electrofocusing. These fractions were tested for LDA to determine the immunoglobulin component responsible for this antibody activity. The subpopulation of lymphocytes responsible for target cell destruction in the LDA reaction was investigated. Peripheral blood lymphocytes were separated on the basis of their T and B receptors and used to determine which population caused target cell destruction.

Major Findings: I - Transplantation Antigens: Several methods were used to prepare soluble cell membrane antigens (HL-A) from tissue culture cell sources. Membrane materials solubilized with nonionic detergents were fractionated to ascertain the molecular characteristics of the HL-A alloantigens. The products were compared with those materials isolated by papain digestion. Immune precipitation tests were developed and used to isolate the HL-A containing membrane substances. The antigen-antibody complexes were dissociated and the antigen characterized on disc gel electrophoresis. Both the detergent solubilized and the papain solubilized products carried the β-2 microglobulin. Size differences in HL-A antigens solubilized by detergents and papain could be accounted for by the action of the enzyme on the native product.

II - Acute Leukemia Associated Antigens: Antiserums obtained from rabbits immunized with the soluble cell membrane component from the cell line RAJI were found to detect an antigen which is associated only with acute leukemia cells. Peripheral white blood cells from patients in remission and from normal peripheral blood lymphocytes could not be demonstrated to contain this antigen. Cultured cell lines derived from various neoplasms were also tested for the presence of this antigen and found to be negative. This antigen could be demonstrated to be induced in human embryonic kidney cell lines coincident with infection with murine Rauscher leukemia virus. This work has been done in collaboration with Dr. Edward Matthews and supported by a contract to Litton Bionetics. Serums obtained from patients with acute leukemic and immunized with the RAJI cell line were bound to leave the same pattern of reactivity as the rabbit strain. These serums were used to separate the leukemia antigens from the HL-A3.

In the studies investigating LDA and serum blocking factors in serums from acute leukemia patients, specific immune reactivity was demonstrated in approximately one-third of the patients. However, in the majority of the patients tested thus far it appears that the serum contains a component which inhibits the reactivity of the LDA activity of heterologous antisera. Other investigators have demonstrated blocking factors in neoplastic disease which may explain the lack of immune reactivity of patients against their developing neoplasms. It appears that there is a blocking factor also in patients with acute leukemia. These studies have been extended to the examination of immune reactivity of leukemia patients immunized with the RAJI cell line. Blocking factors have been demonstrated in the serums of patients during relapse.

III - Characterization of Lymphocyte Dependent Antibody: In studies attempting to isolate and characterize LDA it has been demonstrated that patients receiving multiple transfusions have highest titers of this serum component. These sera were found to react to non-HL-A antigens. Sera were fractionated by gel filtration and ion exchange chromatography as well as electrofocusing. When these fractions were tested it was found that the maximum LDA activity did not correspond with maximum protein concentrations of IgM, IgA, IgG. These findings suggest that the LDA may be a subclass of IgG, or indeed, may be a new class of immunoglobulins. Additional studies have indicated that cytotoxic antibody can be separated from LDA.

Significance to Biomedical Research and the Program of the Institute:
I - Transplantation Antigens: The preparation and analysis of soluble human transplantation antigens has both fundamental and clinical importance. Information as to the character of these antigens and the nature of the integration of these materials into the cell membranes structures will add to our knowledge of genetic control as well as fundamental knowledge in basic cell membrane structure. Such studies using known markers on cell surfaces become the prototype for investigating tumor antigens.

II - Acute Leukemia Associated Antigens: The demonstration that acute leukemia cells possess what appear to be specific antigenic determinants may allow a search for an etiologic agent which will cause this antigen to appear on acute leukemia cells. Immunotherapy has been proposed in the acute leukemias. Such a program implies that acute leukemia cells do possess a particular antigen which is shared by the cells from a number of patients with this disease. Our demonstration that there is indeed an acute leukemia associated antigen makes more rational the treatment of this disease by these means. The demonstration that there are substances in the acute leukemia serum which either block LDA activity or mediate such activity suggests a fine balance between immunity and "tolerance" in the patients with this disease. On the basis of the above observation an immunotherapy program has been initiated. Studies thusfar indicate that patients immunized with RAJI develop antibody to leukemia cells. However this reactivity is lost when the patients disease relapses.

III - Characterization of Lymphocyte Dependent Antibody: The isolation and characterization of the lymphocyte dependent antibody is a relatively new area for exploration. The LDA appears to be an antibody which is not correlated with complement dependent cytotoxic serum components and may thus represent a new mechanism for destruction of tissues by the host's own lymphocytes. Investigation of the nature of this antibody and its specificity of reaction should contribute to our knowledge of immune reactions in patients with autoimmune disease as well as those individuals who possess malignancies.

Proposed Course of Project: Further chemical and biologic characterization of soluble HL-A alloantigens will be undertaken. Studies will be designed to isolate the antigenic determinants by immune precipitation techniques and to characterize this isolated product. Studies will be continued testing solubilized histocompatibility antigen for possible use in clinical situations for the induction of tolerance and/or enhancement.

Studies will continue in the isolation and characterization of the antigen associated with acute leukemia cells. In addition, efforts are currently being made to screen a number of the known oncogenic RNA viruses to attempt to correlate infection with these viruses and the appearance of antigen on cell surfaces. The various factors in the serum from leukemic patients which are either blocking or enhancing cell mediated cytotoxicity reactions will be isolated and characterized.

Further studies will be undertaken in an attempt to identify the specific serum component responsible for the LDA activity.

Honors and Awards: None

Publications:

Mann, D. L., Halterman, R., and Leventhal, B.: Acute leukemia associated antigens. Cancer, in press.

Mann, D. L., Ulmer, C., Pourhiet, A., Leventhal, B., and Halterman, R.: Studies on the immunologic reactivity of serums from acute leukemia patients. Oncology, in press.

Yust, I., Wunderlich, J. R., Mann, D. L., and Terry, W. D.: Identification of lymphocyte dependent antibody in sera from multiply transfused patients. Transplantation, in press.

Springer, T. A., Strominger, J. L., and Mann, D. L.: Partial purification of detergent soluble HL-A antigen and its cleavage by papain. PNAS, in press.

Yust, I., Wunderlich, J. R., Mann, D. L., Rogentine, G. N., Leventhal, B., Yankee, R., and Graw, R.: Human lymphocyte dependent antibody mediated cytotoxicity and direct lymphocyte cytotoxicity against non-HL-A antigens. Nature, in press.

Serial No. NCI-5011(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Preclinical Immunological Studies in Hematopoietic Cell Transplantation

Previous Serial Number: Same

Principal Investigator: John R. Neefe, Jr., M.D.

Other Investigators: Robert G. Graw, Jr., M.D., G. Nicholas Rogentine, Jr., M.D.

Contractor: Litton Bionetics Research Laboratory

Contract N01-CB-02103

Man Years:

Total:	1 1/4
Professional:	1 1/4
Other:	0

Project Description:

Objectives: 1) To investigate newer forms of immune suppression of allograft rejection and the graft-versus-host reaction (GVHR) in a rhesus monkey bone marrow transplant model prior to clinical use; 2) To identify monkey transplantation antigens; 3) To establish a colony of pedigreed rhesus families to study the genetics of transplantation antigens and to eventually provide a source of related donor-recipient pairs for bone marrow transplantation.

Almost all of this work is being conducted by a close collaboration with Litton Bionetics under contract N01-CB-02103. Dr. Darrow is the Principal Investigator and Dr. Rogentine the Project Officer. Dr. Neefe and Dr. Graw are Co-Project Officers.

Methods Employed: Experiments done in the past 12 months have employed a variety of techniques and methods. Femoral bone marrow obtained by aspiration from anesthetized donors is administered intravenously to lethally X-irradiated recipients. Such allografted monkeys are then medically supported and observed for the remainder of their life with frequent measurement of a number of clinical, biochemical, and immunologic parameters. Lymphocyte antigens of the Rhesus monkey are studied by standard eosin dye lymphocytotoxicity tests employing Rhesus alloantisera obtained from planned immunizations with skin grafts and leukocyte injections. The mixed leukocyte culture technique was adapted for use with monkey leukocytes and provides another method of assessing histocompatibility.

The production of anti-rhesus lymphocyte sera (ALS) has continued with the immunization of rabbits with purified rhesus thoracic duct lymphocytes. The ALS resulting from these immunizations has been tested for activity in vitro, and for immunosuppressive properties in vivo using rhesus skin graft and GVH systems.

Major Findings: The major findings of the past year are well detailed in the contractor's Progress Report dated February 1974. We will summarize these findings:

1) Newer Modes of Immune Suppression of Allograft Rejection: In order to facilitate bone marrow takes it was desirable to find the maximal tolerated dose of total body irradiation. Studies involving administration of X-ray in graded doses to monkeys with and without bone marrow autografts demonstrated this dose to be 1050 rads. The influence of bone marrow autograft dose upon survival was then studied. Bone marrow autograft doses greater than 2.5x10(7) per KG permitted hematopoietic reconstitution of the lethally irradiated host in every case. Tempo of recovery was dose dependent and animals receiving the largest grafts enjoyed the shortest period of vulnerability associated with thrombocytopenia and granulocytopenia. The dose dependent pattern of reconstitution with autologous bone marrow permitted study of the hemopoietic stem cell toxicity of anti-lymphocyte serum and it was demonstrated that all animals receiving ALS and X-ray conditioning were restored hemopoietically even by relatively small doses of autologous bone marrow. Thus myelotoxicity was not an important property of the pool of ALS assayed in this test system. The immunosuppressive potential of ALS intended for human use has successfully been assayed in rhesus monkeys and it is quite likely that this model for assay of hemopoietic stem cell toxicity of antirhesus ALS may be useful for assay of ALS for human use. A study of the influence of X-ray dose and bone marrow dose upon engraftment of allogeneic bone marrow and upon the severity of the subsequent GVHR has been initiated. Graft failure and absence of GVHR are seen at 800 rads. At 900-1200 rads animals die sooner than X-ray controls, as had been found in previous years; X-ray dose and bone marrow dose had no influence upon this phenomenon. However, animals receiving large dose of bone marrow-10(9) per KG-often showed earlier engraftment; in addition the histologic lesions of GVHR were more florid although GVHR was not clinically more severe. Completion of planned experiments in this area will permit resumption of studies of ALS for conditioning of allograft recipients under conditions better controlled than previously.

2) Rhesus Histocompatibility Antigens: 150 unrelated and pedigreed

related monkeys supported by this project were typed by other major rhesus typing teams in a primate histocompatibility workshop in September, 1973, in Atlanta and results of this workshop are now being analyzed by the hosts of the workshop at Duke University. Five to fifteen new rhesus histocompatibility antigens will probably be identified as a result of this effort. Antisera previously raised under this project have been subjected to a modified factor analysis which revealed that most sera contain more than one specificity, including usually one of the previously defined specificities. It has been possible to analyze the second and sometimes even the third specificity in these sera and 10 new candidate specificities have been identified in our sera in this fashion. Correlation of these specificities with the new workshop specificities has not yet been undertaken.

3) Development of a Pedigreed Breeding Colony: Several new births in the pedigreed families occurred and plans to add new breeders for the production of 1/2 and 1/4 sibs were initiated.

Significance to Biomedical Research and the Program of the Institute: Considerable resources of the NCI have been devoted to clinical bone marrow transplantation in leukemia and aplastic anemia. Many problems have become apparent with increased clinical experience in this area and these cannot often be studied readily in patients. For instance in vivo validation of in vitro techniques for definition of bone marrow stem cell toxicity of agents such as ALS can be accomplished only in animal models. Only HL-A matched donors have been used for clinical bone marrow transplantation. This greatly limits the application of this form of therapy, as well over 60% of all potential recipients do not have matched donors. Transplantation of mismatched marrow has almost invariably resulted either in no graft take or fatal GVHR and even in perfectly matched donor-recipient combinations fatal GVHR may occur, although at a lower frequency. Our recent findings with respect to the modification of GVHR with ALS and RhL-A matching provides tools for study of the basic mechanism of GVHR and also may be applicable clinically, particularly in situations where it is necessary to transplant marrow between individuals not perfectly matched at HL-A. An understanding of the pathophysiology of GVHR obtained in this system will be of benefit in planning new methods of intervention in this problem as it occurs in man. Marrow transplantation may have a role in tumor immunotherapy but understanding of GVHR is certainly a prerequisite to this.

Proposed Course of Project: The current efforts to improve the usefulness of the rhesus model of bone marrow transplantation by improving the incidence of engraftment through the use of the combination of ALS and X-ray under optimized conditions will be continued. Factors influencing engraftment will then be analyzed. Efforts to correlate clinically important properties of ALS preparations used with in vitro assays will continue. The use of post grafting ALS will be studied for its ability to abrogate clinically manifest GVHR. A new conditioning regimen combining cyclophosphamide and X-ray has been used in human transplantation with considerable attendant toxicity and this regimen will be studied in monkeys in order to alter the dosage and scheduling for maximal tumor kill and immunosuppression and minimal extramedullary toxicity.

Further characterization of the RHL-A system of rhesus histocompatibility antigens will be undertaken. Monospecific sera will be sought through immunizations within family groupings. Typing of random unrelated monkeys as well as retyping of family monkeys with new sera will be performed to find new correlations.

Honors and Awards: None

Publications:

Neefe,J. R., Jr., Merritt, C. B., Vaal, L., Darrow, C. C., II, and Rogentine, G. N., Jr.: Histocompatibility and bone marrow transplantation between unrelated rhesus monkeys. Transplantation 16: 365, 1973.

Neefe, J. R., Jr.: Lack of myelotoxicity of rabbit antilymphocyte serum used in primate bone marrow transplantation. Fed. Proc., in press.

Neefe, J. R., Jr., Graw, R. G., Jr., Darrow, C. C., II, Whang-Peng, J., and Rogentine, G. N., Jr.: Engraftment in ALS-pretreated rhesus monkey recipients of allogeneic bone marrow. J. Reticuloendothelial Soc., in press.

Neefe, J. R., Jr., Merritt, C. B., Darrow, C. C., II, and Rogentine, G. N., Jr.: Beneficial influence of limited histocompatibility of bone marrow grafted to unrelated rhesus monkeys preconditioned with X-ray and ALS. Trans. Proc., in press.

Serial No. NCI-5014(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Lymphocyte Stimulation: The Chemical Basis of Gene Activation in Cellular Immunity

Previous Serial Number: Same

Principal Investigator: Ronald H. Schwartz, M.D., Ph.D.

Other Investigators: Barry S. Handwerger, M.D.

Cooperating Units: None

Project Description:

During the course of this project a new technique was developed for separating thymus-derived and bone marrow-derived lymphocytes. The potential use of this procedure was so great that a decision was made to terminate this project (NCI-5014(c)) in favor of exploiting fully the new technique (see NCI-5039(c)).

Serial No. NCI-5016(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cellular Immunity II

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Dean L. Mann, M.D.

Contractor: Litton-Bionetics Research Laboratory

Contract N01-CB-02207

This progress report represents only a portion of work supported by Contract N01-CB-02207

Cooperating Units: Michael Blaese, M.D., Metabolism Branch, NCI;
Howard Dickler, M.D., Immunology Branch, NCI;
Ronald Schwartz, M.D., Ph.D., Barry Handwerger, M.D., Immunology Branch, NCI
John Potter, M.D., Georgetown University

Man Years:

Total:	3 1/2
Professional:	1/2
Others:	3

Project Description:

Objectives: This project, which was initiated in 1970, has been directed primarily at understanding cell-mediated immune cytotoxicity in humans. The long-range goals are: 1) to identify human cells capable of destroying foreign cells by cytotoxic, cell-mediated immune reactions, 2) to characterize factors which influence development of cellular immune effector cells, 3) to characterize mechanisms of foreign cell destruction by effector cells, and 4) to define changes occurring in the cellular immune cytotoxic responses which accompany immune deficiency diseases, malignancies, transplanted organs, autoimmune states, and iatrogenic immune suppression.

A year and a half ago the project was expanded to include animal model systems directly relevant to the human work. The primary goal of the animal model system work is to determine if syngeneic tumors grow because of an inadequate or an inappropriate immune response.

Methods Employed: 1) *Human Direct, Cell-Mediated Immune Cytotoxicity*. This cytotoxic pathway is measured by the release of ^{51}Cr from labeled target cells incubated with immune cells for 4 hours. Details of the assay utilizing human cells have been described elsewhere (Wunderlich, et al., *Transplantation* 13:31, 1972). Target cells are prepared from peripheral blood, pathological fluid exudates, tissue culture lines, and biopsy specimens of lymph nodes and solid tumors. Solid tumor samples and cells adhering to tissue culture surfaces are mechanically separated. Aggregated cells are allowed to settle. Red cells are then removed by ammonium chloride Tris lysis. The resulting cell suspension is labeled with ^{51}Cr. Normal and immune lymphoid-attacking cells are prepared primarily from human peripheral blood. Heparinized peripheral blood is passed through nylon columns to remove polymorphonuclear cells and monocytes and then settled in plasmagel to reduce the red blood cell content. Alternatively, heparinized peripheral blood has been fractionated on Ficol-hypaque and subsequently incubated on nylon columns. The latter procedure is highly effective for removing adherent cells. Studies of multiply transfused humans have shown that cytotoxicity in this assay is specific for direct cell-mediated reactions and independent of conventional complement, humoral antibody, and nonspecific toxins.

2) *Human Lymphocyte Dependent Antibody (LDA) Mediated Cytotoxicity*. This cytotoxic pathway is also measured by a chromium release assay. After ^{51}Cr labeling, target cells are washed by centrifugation and then incubated for 1/2 hour with heat inactivated serum or plasma containing LDA. Incubation mixtures include carrageenan, a C1 attachment inhibitor. Target cells are again washed and then incubated with nonsensitized attacking lymphoid cells for 4 hours. In one set of experiments attacking cells have been pretreated with aggregated human immunoglobulin using the technique of Dickler and Kunkel (*J. Exp. Med.* 136:191, 1972). In a second set of experiments directed at testing the effect of lymphocyte activation on effector cell activity, peripheral blood lymphocytes have been maintained in vitro for 5-6 days prior to cytotoxicity testing. During this period lymphocytes have been activated by mitogens or by mixed lymphocyte reactions (see below).

3) *In Vitro Immunization of Human Peripheral Blood Lymphocytes*. Human lymphocytes have been immunized against allogeneic cells using the procedures of Miggiano and his colleagues (*Transplant. Proc.*, 4:231, 1972). This procedure has been modified by incorporating several in vitro immunization techniques of Mishell and Dutton (*J. Exp. Med*. 126:423, 1967), which have been highly successful in mouse culture systems.

4) *In Vitro Cytotoxicity Testing for Melanoma Patients on the NCI Cooperative Melanoma Immunotherapy Protocol*. Peripheral blood samples from patients in the Immunotherapy Protocol are being evaluated for direct cell-mediated cytotoxicity (see above), lymphocyte-dependent antibody (see above), and complement-dependent antibody. The allogeneic melanoma line used for clinical

immunization is also being used as a target cell in these assays. The complement assay is a two-stage, ^{51}Cr-microcytotoxicity test utilizing rabbit complement.

5) Tumor Growth Resulting from Inadequate or Inappropriate Immune Responses in a Mouse Model System. Various parameters of the immune response are being monitored in BALB/c mice injected with a strain of Moloney sarcoma virus which leads, for the most part, to progressively growing, ultimately fatal tumors. Immune parameters being assessed are (a) direct cell-mediated cytotoxicity, (b) lymphocyte dependent antibody, (c) blocking factors, (d) complement dependent antibody, and, (e) memory for direct cell-mediated cytotoxicity. Unique aspects of this project involve the use of freshly explanted and subsequently fractionated tumor cells as target cells and freshly explanted lymphoid cells as attacking cells. We feel this source of reagent cells best represents in vitro the ability of the host to recognize and damage specific tumor cells. An inappropriate immune response is defined here by the absence of cytotoxic lymphocytes and humoral antibody, blocking factors (cells or antibody) or memory for direct cell-mediated cytotoxicity, however, are present. An insufficient immune response is defined here by the presence of cytotoxic cells and/or antibody and the lack of blocking factors.

Major Findings: (1) Human Lymphocyte Dependent Antibody (LDA). We have further investigated the nature of human lymphocytes which cooperate with LDA to induce target cell damage. Two lines of evidence argue that B lymphocytes from peripheral blood of normal human donors can destroy antibody sensitized target cells: (a) effector cell function of lymphocytes from hypogammaglobulinemic patients is significantly reduced compared to the function of lymphocytes from normal donors, and (b) pretreatment of purified lymphocytes with heat aggregated immunoglobulin inhibits effector cell function. Results from the latter line of experiments additionally argue that subclasses of B lymphocytes exist which cooperate with LDA. This conclusion is supported by immunoglobulin aggregate dose dependent studies which reveal discreet plateaus in the inhibitionof effector cell activity. The possibility that "activated" lymphocytes also serve as effector cells in lymphocyte dependent antibody mediated cytotoxicity has been investigated by mitogen pretreatment of effector cells in vitro. Peripheral blood lymphocytes pretreated with phytohemagglutinin, Concanavalin A or pikeweed mitogen are more cytotoxic than nontreated controls. This effect appears not to be a result of nonspecific cytotoxicity associated with mitogen activation, a result of media conditioning by mitogens or a result of mitogen carryover to the cytotoxicity assay. In addition, lymphocytes have been specifically activated in vitro by a mixed lymphocyte reaction. These activated lymphocytes are directly cytotoxic for sensitizing target cells when compared to lymphocytes cultured without allogeneic stimulating cells. They also display, however, increased cytotoxicity against antibody sensitized syngeneic target cells in an LDA reaction. These findings argue that activated human peripheral blood lymphocytes activated either nonspecifically by mitogens or specifically in a mixed lymphocyte reaction are more cytotoxic than nonactivated effector cells in LDA reactions.

(2) In Vitro Immunization of Human Peripheral Blood Lymphocytes. The nature of direct cell-mediated cytotoxicity in humans is also being pursued by use of in vitro immunization techniques. Leukocytes from peripheral blood of normal donors have been successfully sensitized in vitro against allogeneic leukemic blast cells which have been stored in liquid nitrogen and also against an allogeneic human cell line known to share an antigen with acute leukemia cells.

(3) In Vitro Testing of Patients on the Melanoma Immunotherapy Protocol. In vitro cytotoxicity tests have been established in order to monitor the response of melanoma patients to an allogeneic melanoma cell lines. The same melanoma line (RPMI-80 2) as that being used for immunotherapy is also being used as a target cell for direct cell-mediated cytotoxicity, lymphocyte dependent antibody and complement dependent antibody tests. Both complement dependent antibody and lymphocyte dependent antibody tests have detected cytotoxic antibody in patients following immunotherapy. Tests for direct cell mediated cytotoxicity using patient's lymphocytes as attacking cells have been negative. It is important to note the positive responses may be against alloantigens as opposed to tumor associated antigens.

(4) Tumor Growth Resulting From an Inadequate or Inappropriate Immune Response in a Mouse Model System. Murine sarcomas induced by a Moloney sarcoma virus in BALB/c mice have been used for this study. Fractionation fo cells dispersed from freshly explanted solid tumors yields tumor cells suitable for use as target cells in our ^{51}Cr release cytotoxicity assays. Thus, separation of mechanically dispersed tumor cells on discontinuous BSA density gradients yields cells on the most buoyant gradient layers which are enriched for viability and tumor cell content as detected by both morphology and tumor transfer tests. Cell-mediated cytotoxic activity against syngeneic MVS tumors has been detected using spleen cells from which most B lymphocytes, monocytes and macrophages have been removed by nylon column filtration. Nylon column fractionated spleen cells from mice which had rejected tumors have shown the highest anti-tumor activity. Nylon column fractionated spleen cells from mice with progressively growing tumors have shown the lowest anti-tumor activity. Although this finding is intriguing, the nature of the reaction is unclear in that nylon column fractionated spleen cells from "normal" mice are also cytotoxic for MSV-induced tumor cells.

Significance to Biomedical Research and the Program of the Institute: (1) Human Lymphocyte Dependent Antibody (LDA) · LDA mediated cytotoxicity provides a mechanism by which normal lymphoid cells can be utilized for target cell destruction. This is important for tumor rejection, since many of the host lymphoid cells attracted to reaction sites may be nonsensitized cells. Not all host lymphoid cells, however, appear to be capable of mediating LDA cytotoxic reactions. Thus it is important to identify those host cells which can cooperate in a cytotoxic reaction, if we hope to assess the antitumor properties of host cells which infiltrate a tumor. Not only is the class of lymphocytes important (T versus B lymphocytes, etc.), but also the state of cell activation.

(2) In Vitro Immunization. Recent studies have identified several host factors which will lead to a suboptimal cellular immune response against tumor antigens in situ, e.g., (a) suboptimal ratios of immunizing tumor antigens and immunocompetent cells (b) active development of suppressor cells or humoral blocking antibody, and (c) buried tumor antigens on tumor cells. This list is growing as further observations are made on host immune responsiveness. These detrimental influences on the host immune response can, to a large extent, be bypassed in vitro, and hopefully strong immune responses against tumor antigens will result. Such results would encourage not only the study of immune lymphocytes specifically cytotoxic for human tumor cells, but also lead to effective passive immunotherapy.

(3) In Vitro Testing of Patients on the Immunotherapy Protocol. In vitro cytotoxicity tests are being used to provide one parameter for assessing the effectiveness of patient immunization with melanoma cells. Although these tests probably detect patient responsiveness to allogeneic transplantation antigens, they may also serve the useful purpose of detecting nonresponsive patients or the presence of an ineffective vaccine.

(4) Immunologic Monitoring of Hosts with Progressively Growing Tumors. A uniquely significant aspect of this work is the use of freshly explanted purified tumor cells as target cells in rapid ^{51}Cr release assays. This approach minimizes deviation of either attacking cells or tumor cells from their behavior in situ. In our opinion, previous studies utilizing 2-3 day in vitro assays demonstrate sensitization of the host but not the existence of cytotoxic immune cells in situ. For example, cytotoxic precursor cells, whose further maturation is blocked by an inhibitory host environment, may differentiate into cytotoxic cells during the in vitro tests. In addition, target cells which either are tissue culture lines or tumor cells which have been passaged in vivo for many generations may not be similar in antigenicity or destructability to tumor cells as they exist in situ in the primary host.

Proposed Course of Project: (This subject matter is presented in more detail in the 1974-75 renewal proposal for NCI Contract No. NO1-CB-02207.) (a) Continue to identify subpopulations of human lymphocytes which damage antibody sensitized target cells and explore the mechanism by which activation of attacking lymphocytes enhances their cytotoxic effector cell activity against antibody sensitized target cells. (b) Attempt to improve the in vitro development of cytotoxic effector cells by fractionating responder cells. (c) Continue to evaluate patients on the melanoma immunotherapy protocol for humoral and cellular cytotoxic immune responses as reflected by the presence of direct cell mediated cytotoxicity, complement dependent antibody and lymphocyte dependent antibody. (d) Continue to evaluate the immune responsiveness of mice bearing progressively growing MSV-induced tumors.

Honors and Awards: None

Publications: None

Serial No. NCI-5017(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of the Biological Significance of Cell Surface Antigens with Emphasis on Antigens Revealed by Neuraminidase Treatment and on Histocompatibility Antigens

Previous Serial Number: Same

Principal Investigator: G. Nicholas Rogentine, Jr., M.D.

Other Investigators: Enser W. Cole, III, M.D.

Cooperating Units: Warren Strober, M.D., NCI, Robert Trapani, Ph.D. Microbiological Associates, Inc., Joseph Fraumeni, M.D., NCI, Paul Chretien, M.D., NCI, John Whitaker, M.D., University of Tennessee, Memphis

Man Years:

Total:
Professional:
Other:

Project Description:

Objectives: Definition of the natural biological significance of certain human cell surface antigens is our objective. We have studied two groups of antigens. The first is an antigen or set of closely related antigens revealed after neuraminidase treatment of intact cells. The second group is the well known HL-A antigen system. Nothing as yet is known of the biological significance of the first group of antigens. The latter group has importance for organ transplantation, but our emphasis is upon the relationship of HL-A antigens to the natural function of the human organism.

We are approaching the significance of the neuraminidase revealed antigen(s) by studying the chemical nature of the antigen, its distribution on different cells, and its naturally occurring autoantibody.

We are seeking the significance of HL-A antigens by investigating their frequency disturbances in different diseases. If such disturbances are found, and many have been, we can design further studies on the role of the antigen in the pathophysiology of disease.

Methods Employed: Cell surface antigens are assayed by standard ^{51}Cr release or dye exclusion cytotoxicity tests. Cells, usually lymphocytes, are either untreated or incubated with Vibrio cholera neuraminidase under conditions which do not effect cell viability but which remove most of the cell surface sialic acid. Estimates of antigen content of different cells are achieved by quantitative inhibition of antisera. Carbohydrate hapten inhibition experiments were used to define the antigenic determinant(s) on neuraminidase treated cells. HL-A typing is being conducted on contract by Dr. Robert J. Trapani, Microbiological Associates, Inc.

Major Findings: In the past year we concentrated on studies of the neuraminidase revealed antigen(s) on human lymphoid cells. Hapten inhibition experiments with chemically defined oligosaccharides revealed that all of 8 natural antibodies tested were inhibited by carbohydrates containing nonreducing end β-D-galactopyranosyl residues. There appeared to be antibody heterogeneity, for some sera were inhibited best by lacto-N-neotetraose (Galβ1-4Glc NAcβ1-3Galβ1-4Glc), others by Gal 1-3GalNAc. Moreover, human lymphoid tissue culture cells appeared to contain both antigens, whereas peripheral blood lymphocytes bore only the Galβ1-3GlcNAc determinant.

Tissue culture T lymphoblasts and acute lymphocytic leukemia lymphoblasts (presumably T cells) have little neuraminidase revealed antigen in contrast to tissue culture B lymphoblasts and peripheral blood lymphocytes (mature B and T cells). Human erythrocytes of all common ABO and MN phenotypes bear the antigen also.

A naturally occurring cytotoxic antibody possessed by most normal humans reacts with the neuraminidase revealed antigen. This antibody lyses autologous as well as allogeneic lymphocytes. It is complement dependent and does not lyse untreated cells. Last year we studied the antibody in greater detail. We found it in the IgM fraction in 6 of 6 sera tested. Heating serum for 30' at 56°C did not inactivate it, unlike many other natural antibodies. It lysed lymphoid cells at 15°C and 26°C, but not at 4°C or 37°C.

Although over 85% of normals tested had detectable antibody, there was marked variation in amounts. Currently we are studying the reasons for this variation. We are also assaying antibody levels in patients with immune deficiencies and immune abnormalities looking for groups of individuals with abnormally high or low antibody levels. Thus far patients with a- or hypogammaglobulinemia (as expected) and the Wiskott-Aldrich syndrome have no detectable antibody. We are also studying antibody levels in patients with malignancy. We have hypothesized that these antibodies are antitumor antibodies and represent part of an immune surveillance system. Therefore, patients with newly diagnosed malignancies should have low levels of antibody.

Studies of the association of HL-A antigens and disease concentrated on four areas last year.

Clearer definition of the association of HL-A8 with dermatitis herpetiformis (DH) and gluten sensitive enteropathy (GSE) was achieved. 90% of patients with GSE have HL-A8. The normal frequency is 22%. 60% of DH patients have HL-A8. About 2/3 of patients with DH have atrophic intestinal mucosae similar to that seen in GSE. In DH patients with gut pathology, the HL-A8 incidence is about 90%, whereas in DH patients without gut pathology the incidence is normal. HL-A8 appears to be associated with the gastrointestinal pathology seen in GSE and DH.

With Drs. John Whitaker and Sidney Stein we have studied about 60 patients with multiple sclerosis. We found a slight but significant increase in HL-A7 in this group. Currently, antibody titers to measles and other viruses implicated in the disease pathogenesis are being obtained and correlated with HL-A type.

With Dr. Paul Chretien we are determining HL-A antigen frequencies on a large number of patients with squamous cell carcinoma of the cervix, head and neck, lung, and esophagus. We are also typing patients with melanoma and families having many members with cancer. These studies are in progress.

Finally we found a slight increase in HL-A13 frequency in patients with pemphigus.

Significance to Biomedical Research and the Program of the Institute: Cell surface molecules are involved in important recognition phenomena controlling normal growth and development. Malignancies arise when this recognition mechanism fails. Thus the study of these molecules is of critical importance to understanding normal growth and development and malignancy. Fortunately many cell membrane structures are antigenic, allowing identification by standard immunologic techniques. It is our purpose to study selected plasma membrane antigens and their respective antibodies.

Neuraminidase treated tumor vaccines are being widely used in human and animal tumor immunotherapy. An understanding of the immunological nature of the cell surface antigenic change following neuraminidase treatment is essential to the rational application of this promising mode of therapy. Moreover, the study of the natural autoantibodies to the neoantigen may be of great importance in understanding natural cell death or mechanisms of immune surveillance.

The cell surface histocompatibility antigens are a product of an extremely polymorphic genetic system which must have important survival value for the individual irrespective of its role in allograft rejection. For example, the possession of a given HL-A specificity may confer, by mechanisms unknown, susceptibility (or resistance) to a particular disease. Therefore, one rational approach to assessing the natural biological significance of HL-A antigen is the study of the association of disease with these antigens. If such associations are found, they have double significance. Closer medical observation of individuals possessing disease-associated HL-A antigens may result in earlier diagnosis and better control of the disease. In addition such knowledge may help us design experiments to probe the disease's basis pathophysiology.

Proposed Course of Project: Further studies of the antigen revealed by neuraminidase treatment of human lymphocytes will focus on the immunochemical nature of the antibody, possible abnormalities of antibody levels in disease, and on the biological significance of this pehnomenon. A variety of patients with diseases, especially malignancies and disorders of immunity, will be typed for HL-A antigens. Correlations will be sought and experiments designed to probe the pathogenesis of the disease will be attempted.

Honors and Awards: None

Publications:

Rogentine, G. H., Trapani, R. -J., Yankee, R. A., and Henderson, E. S.: HL-A antigens and acute lymphocytic leukemia. The nature of the HL-A2 association. Tissue Antigens 3: 370-476, 1973.

Graff, K. S., Simon, R. M., Yankee, E. A., DeVita, V. T., and Rogentine, G. N.: HL-A antigens in Hodgkin's disease. J. Nat. Cancer Inst., in press.

Whisnant, J. K., Rogentine, G. N., Jr., and Robbins, J. B.: Studies on mechanism of susceptibility to H. influenzae type b disease. Relationship of erythrocyte and histocompatibility antigens with H. influenzae type b meningitis and epiglottitis. In H. influenzae, ed. S.H.W. Sell and D. T. Karzon. Vanderbilt University Press 1973, p 197-202.

Everson, L. K., Plocinik, B., and Rogentine, G. N., Jr.: HL-A expression on the G_1, S and G_2 cell cycle stages of human lymphoid cells. J. Nat. Cancer Inst., in press.

Katz, S. I., Dahl, M. V., Pennys, N., Trapani, R. J., Rogentine, G. N.: HL-A antigens in Pemphigus. Arch. Derm. 108: 53-55, 1973.

Gebhard, R.L., Katz, S. I., Marks, J., Shuster, S., Trapani, R. J., Rogentine, G. N., Strober, W.: HL-A antigen type and small intestinal disease in dermatitis herpetiformis. Lancet II, 760-762, 1973.

Serial No. 5018 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization of Cell Surface Molecules on Lymphoid Cells

Previous Serial Number: NCI 5018(c)

Principal Investigator: Pierre A. Henkart, Ph.D.

Other Investigators: Richard I. Fisher, M.D.; Howard B. Dickler, M.D.;
Elaine L. Alexander, M.D., Ph.D.

Man Years:

Total:	3 1/2
Professional:	2 1/2
Other:	1

Project Description:

Objectives: To identify and characterize several functionally important lymphocyte cell surface molecules. These include (1) the glycoproteins which are the surface receptors for the mitogens concanavalin A (Con A) and phytohemagglutinin (PHA) and (2) the Fc receptor and other cell surface molecules involved in lymphocyte-mediated cytolysis.

Methods Employed: (1) Cell surface proteins were labeled by the technique of iodination with radioactive iodine catalyzed by lactoperoxidase. Solubilization of the cell membrane was carried out with nonionic detergent, followed by addition of the mitogen to bind its receptor; the receptor-mitogen complex was precipitated by specific antimitogen antibody and washed. These precipitates were dissolved with SDS and run in SDS gel electrophoresis.
(2) To identify the Fc receptor (in collaboration with Dr. Howard Dickler) we have used this iodination and solubilization technique to attempt to identify a component specifically binding to aggregated γ globulin. In vitro lymphocyte dependent antibody-mediated cytolysis has been studied using ^{51}Cr labelled human red cells modified with the TNP hapten. Characteristics of the Fc receptor have been defined in these studies, since the Fc receptor appears to provide the means of attachment of the target cell to the attacker cell. We have tested various types of anti-TNP antibodies. We have also modified the cell surface of the attacking lymphocytes.

Major Findings: (1) Human peripheral blood lymphocytes contain many surface glycoproteins which bind PHA and Con A. Con A binds more surface label than PHA, but the labeled receptors have very similar gel profiles, including the four major receptor proteins. We have concluded that both mitogens bind to the same set of membrane receptors.
(2) Antibody coated human TNP red cells and TNP lymphocytes are lysed very efficiently by non-immune human lymphocytes during a short term culture. This lysis is not mediated by phagocytic cells. IgG anti-TNP sensitizes the targets efficiently, while we have been unable to demonstrate killing with IgM anti-TNP. A TNP-binding mouse IgA myeloma protein also does not sensitize the targets but does block subsequent coating and lysis by IgG. Reduction and alkylation of IgG anti-TNP causes a loss of lymphocyte-dependent lytic activity while the hapten binding is unaffected.

Pretreatment of the attacking lymphocyte with trypsin under conditions where the Fc receptor is unaffected causes a loss in their killing activity. This implies that another lymphocyte cell surface component may be important in the killing process.

Significance to Biomedical Research and the Program of the Institute: Basic general knowledge of cell surface molecules of higher organisms is still rudimentary. At present it is unclear how many different molecules are exposed to the exterior and how they mediate their many functions of recognizing external molecules and other cells. Lymphocyte mediated cell killing is thought to be the principal process responsible for the immunological rejection of grafts and tumors, yet the basic mechanism for this killing is unknown. The present evidence suggests a hypothesis that lymphocyte surface molecules first recognize the target cell, that other lymphocyte surface molecules may become activated to produce a substance which disrupts the membrane of the target cell. If proved, this hypothesis would provide a basic understanding of a process which is fundamental to the process of immunological rejection of tumors.

Proposed Course of Project: Further efforts are being made to define the specificity of the Fc receptor and to confirm that the Fc receptor is involved in the adherence of attacker and target in antibody-mediated lymphocyte dependent cytolysis.

We have begun a collaborative study in which we will observe lymphocyte mediated killing in the phase microscope while monitoring the membrane resistance of an individual target cell with a microelectrode. We will be able to see if membrane ionic permeability breakdown precedes other morphological events indicating cell death. By fixing at various times after the decrease in membrane resistance we will be able to obtain an ultrastructural record of the events in cell death, possibly leading to new information on the killing mechanism.

The use of chemically modified syngeneic cells in the in vitro induction of cytotoxic lymphocytes will be studied in collaboration with Dr. Gene Shearer, using a new reagent, carboxypyridine disulfide, which modifies cell surface SH groups.

Honors and Awards: None

Publications:

Serial No. NCI-5019 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structural and Immunochemical Analysis of Membranes of Normal and Malignant Cells and of Products of These Cells

Previous Serial Number: Same

Principal Investigator: William D. Terry M. D.

Other Investigators: Pierre Henkart, Ph.D.

Cooperating Units: Charles W. Todd, Ph. D., City of Hope Hospital, Duarte, California; David Goldenberg, M.D., University of Kentucky, Lexington, Kentucky.

Man Years:

Total:	1/2
Professional:	1/2
Others:	

Project Description:

Objectives: Attempts will be made to isolate and characterize membrane proteins or cell products which (1) appear to be associated with the malignant state, and/or (2) appear to be important in acquiring an understanding of membrane structure, and (3) appear to have functional significance in the immune response.

Methods Employed: A variety of procedures are used to solubilize membranes, followed by isolation of protein components by gel filtration, column chromatography, isoelectric focusing and other forms of preparative electrophoresis. Isolated proteins are assessed for purity by acrylamide gel electrophoresis, and immunochemical techniques. Amino acid compositions of proteins and enzymatically prepared fragments are determined on the amino acid analyzer. Sequence analysis is carried out with manual and automatic sequencing procedures. Immunochemical studies utilize complement dependent cytolysis assays, and radioimmunoassays.

Major Findings: Additional studies of carcinoembryonic antigen have shown that CEA isolated from the circulation of a patient with metastatic colon cancer has the same amino terminal sequence as the material isolated from hepatic metastases. In addition, the same sequence has been determined for "CEA" isolated from human colon tumors grown in hamsters.

Significance to Biomedical Research and the Program of the Institute: Demonstration of identical N-terminal amino acid sequences of CEA isolated from metastases to the liver, colon tumor transplants and peripheral blood establishes conclusively that the molecule being analyzed chemically is the same molecule detected in the radioimmunoassay.

Proposed Course of Project: Additional structural studies of CEA and membrane proteins will be carried out.

Honors and Awards: None

Publications:

Coligan, J. E., Henkart, P. A., Todd, C. W. and Terry, W. D.: Heterogeneity of the carcinoembryonic antigen. Immunochemistry 10: 591-599, 1973.

Terry, W. D., Henkart, P. A., Coligan, J. E. and Todd, C. W.: Carcinoembryonic antigen: Characterization and clinical applications. *Transpl. Rev.* 20: 100, 1974.

Terry, W. D., Henkart, P. A., Das, S., Coligan, J. E. and Todd, C. W.: Characterization of human carcinoembryonic antigen. *In* Proc. Miles Seventh International Symposium. Baltimore, Johns Hopkins, 1973 in press

Serial No. NCI-5021(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Antigens Determined by the Major Histocompatibility Locus

Previous Serial Number: Same

Principal Investigator: David H. Sachs, M.D.

Other Investigators: C. Garrison Fathman, M.D.

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives: 1) To produce antibodies against defined regions of the major histocompatibility complex. 2) To characterize the reactions of these antibodies with the cell surface of lymphocytes, and to determine the nature of the cells bearing individual antigens. 3) To attempt to produce anti-idiotypic antibodies against the receptors on these antibodies which detect cell surface histocompatibility antigens.

Methods Employed: Congenic resistant strains of mice differing only at their major histocompatibility loci have been reciprocally immunized in order to produce antibodies of known, restricted specificity. The antibodies have been characterized by assays of complement-mediated lymphocytotoxicity and by fluorescence microscopy using a fluoresceinated rabbit antimouse immunoglobulin as a developing reagent.

The presence of unexpected specificities in these sera reacting with subpopulations of lymphoid cells has led to the development of methods of distinguishing subpopulation reactions. Additivity studies for cytotoxicity reactions and fluorescent staining have been performed with these reagents in combination with reagents of known specificity for B or T subpopulations of lymphoid cells. Also, reactions with fractionated lymphoid cell populations have been performed. T-cell derived tumors have been used for the absorption of the congenic antisera in order to reveal further specificities for antigens expressed preferentially on B cells. In addition, use has been made of congenic resistant animals bearing recombinant H-2 alleles in order to determine the genetic localization of many of the new antigens.

Antibodies against individual antigenic specificities have been isolated by absorption onto lymphoid cells bearing those specificities, and the antibody-coated cells have then been injected back into syngeneic animals in order to immunize against the "receptor" portions of the antibody molecules. Sera have been collected from these animals after multiple immunizations and are being characterized for the presence of antibodies capable of reacting with the appropriate antihistocompatibility antigen receptors of antibodies and cells. Tests include inhibition of cytotoxicity, binding to solid-phase immunoabsorbents, and in vitro and in vivo blocking of the immune response to these antigens.

Similar protocols are also being applied to antibodies raised in inbred rats differing at multiple histocompatbility loci, and to cross-species systems.

Major Findings: 1) Several of the antisera produced in congenic resistant mice have been shown to contain additional specificities to those expected in these strain combinations. These specificities have been shown to be determined by genes mapping within the Ir region of the murine major histocompatibility locus, and to be expressed preferentially on B cells (1). Similar antigenic specificities have simultaneously been described by other laboratories, and the name Ia (Ir associated) antigens has been proposed to describe this new class of cell-surface antigens (2).

2) The discovery of one of these Ia specificities on the B cells of a congenic recombinant mouse differing from another mouse only in the Ir region has been found to correlate with the previously described "one-way" mixed lymphocyte culture (MLC) reaction in this strain combination. This has led to the hypothesis that these Ia antigens may be associated with or responsible for the MLC reaction (3).

3) Anti-idiotypic reactions against the anti-H-2 antibodies have so far been demonstrable only in the presence of anti-allotype reactions and have been extremely weak. Chemical modifications of the anti-H-2 antibodies before immunization may provide a means of increasing the anti-idiotypic reaction.

Significance to Biomedical Research and the Program of the Institute: 1) The new Ia antigens we have found appear to provide a new cell-surface marker for the B subpopulation of lymphocytes. In addition, the localization of the genes determining these antigens to the Ir region may indicate that these B cell surface structures are involved in the T cell-B cell collaboration necessary for many immune responses. If so, these antigens may provide markers for dissecting the mechanism of that collaboration.

2) The specificity of the receptor for histocompatibility antigens should reside in the variable portion of the heavy and light chains of the relevant antibody molecules. Thus, anti-idiotypic antibodies against such receptors might be expected to distinguish those cells capable of reacting against individual histocompatibility antigens. Such antibodies, if obtained, could thus provide a new approach to modification of the immune response to cell-surface antigens.

Proposed Course of Project: 1) Other antisera raised between congenic resistant strains of mice differing by known regions of the major histocompatibility complex will be screened for additional Ia specificities. The cellular distribution of these specificities will be determined (i.e., T cells vs B cells), and lymphoid tumors of known cellular origin will be used to absorb differential activities.

2) Models for the functional properties of these antigens which may explain their genetic localization to the Ir region will be further explored. These will include MLC reactivities, cellular cooperation models and cellular cytotoxicity models.

3) Attempts at immunization against the receptors of anti-H-2 antibodies will be continued. Chemical modification of the constant regions of these antibodies will be attempted in order to overcome the natural tolerance to the carrier portion of the molecules which may explain the necessity for an allotype difference which we have observed.

4) Extensive testing as outlined above will be required in order to determine whether or not anti-idiotypic antibodies have been produced by any of our immunization protocols. If we can demonstrate the presence of such antibodies serologically, we will then attempt to purify them by selective immunoabsorption on columns bearing the appropriate immunoglobulins. The effects of these antibodies on in vitro cellular immune responses (e.e., MLC, cellular cytotoxicity) and on in vivo cellular and humoral responses to tissue grafts will then be tested.

Honors and Awards: None

Publications:

1) Sachs, D. H., and Cone, J. L.: A mouse "B" cell alloantigen determined by gene(s) linked to the major histocompatibility complex. J. Exp. Med. 138: 1289-1304, 1973.

2) Lozner, E. C., Sachs, D. H., Shearer, G. M., and Terry, W. D.: B-cell alloantigens determined by the H-2 linked Ir region are associated with mixed lymphocyte culture stimulation. Science 183: 757-759, 1974.

3) Shreffler, D., David, C., Gotze, D., Klein, J., McDevitt, H., and Sachs, D.: Genetic nomenclature for new lymphocyte antigens controlled by the Ir region of the H-2 complex. Immunogenetics, in press.

Serial No. NCI-5022(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Genetics of the Idiotype of a Human γA Myeloma Protein

Previous Serial Number: Same

Principal Investigator: David H. Sachs, M.D., and Joseph D. Robinson, M.D.

Other Investigators: William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: 1) To determine whether or not the idiotype of human myeloma proteins, as classically defined, also includes restricted allotypes which can be detected in the normal immunoglobulin pools of related individuals. 2) To determine whether or not the idiotype of a human myeloma protein can serve as a TSTA (Tumor Specific Transplantation Antigen).

Methods Employed: A homogeneous preparation of an IgA myeloma immunoglobulin from a 19-year old male was made and used to raise an antiserum in a goat. Selective immunoabsorption of this goat-antihuman serum was performed using tube absorption with pooled normal, maternal, and paternal sera, as well as a column elution from the Sepharose bound IgA immunogen. This antiserum was tested for anti-idiotypic purity against the purified IgA (idiotype) by immunoelectrophoresis and Ouchterlony double diffusion methods. A double antibody radioimmunoassay using I^{125}-labelled idiotype has also been developed to assess nanogram quantities of the idiotype if they persist.

Major Findings: 1) The purified anti-idiotype is an IgG which precipitates on Ouchterlony only with the patient's pretreatment and early treatment period sera, but not with post-treatment sera, a panel of 47 randomly chosen IgA myeloma proteins, nor with maternal, paternal, or normal sera. Thus, when all activity against species and family proteins was removed, there remained an activity against only the original IgA myeloma. 2) The patient remains in clinical remission on intermittent high dose alkeran-prednisone therapy and is without evidence by Ouchterlony double diffusion precipitation of any of the original idiotype in his sera. 3) Using the anti-idiotype as a reagent a double antibody radioimmunoassay has been developed and is presently being used to extend this analysis.

Significance to Biomedical Research and the Program of the Institute: 1) The patient's young age and the availability of both parents as serum donors presents a unique opportunity to study the genetics of idiotype inheritance in man. Since all alloantigens of the patient must have derived from either one or the other of his parents, absorptions of antimyeloma antisera with globulin fractions from the two parents should permit unequivocal distinction between restricted allotypes and true idiotype of the myeloma. 2) If the myeloma idiotype can be shown to also be a TSTA of the tumor cells or of the precursor lymphocytes (of the restricted allotype), then it may provide a marker for tumor specific immunotherapy. 3) The radioimmunoassay provides a system for measuring nanogram subclinical levels of myeloma idiotype in the patient's serum and may serve as a method of following the patient's course and as a guide to further therapy.

Proposed Course of Project: Further characterization of the antimyeloma antibodies will be carried out as outlined above. If restricted allotype is identified, it will be examined in as many family members as possible, both as a humoral specificity and as a possible cell surface antigen.

The anti-idiotypic antibodies we have prepared will be used to screen family members' serum and lymphoid cells. Reactions with the patient's lymphocytes and stored plasma cells will be characterized by fluorescent-labeled antibodies and by cytotoxic reactions.

The radioimmunoassay will be used to screen serial samples of the patient's sera as well as those of family members, making quantitative determinations of idiotype levels. The anti-idiotype has been labeled with fluorescein to test for the presence of idiotype on the surface and within frozen marrow cells from the patient. Ultimately, such an antiidiotypic serum might be used to target on the idiotypic cell marker as a tumor specific transplantation antigen.

Honors and Awards: None

Publications: None

Serial No. NCI-5023 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Transplantation Antigens of Swine

Previous Serial Number: NCI-5023

Principal Investigator: David H. Sachs, M.D.

Other Investigators: None

Cooperating Units: NIH Animal Center, Poolesville, Md.

Man Years:

Total:	3/8 year
Professional:	1/8 year
Others:	1/4 year

Project Description:

Objectives: To develop at least two strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (SL-A). The animals will subsequently be used for in vivo experiments in organ and tissue transplantation and as a source of large numbers of cells from which cell surface antigens can be isolated, purified and characterized chemically.

Methods Employed: Unlike classical inbreeding schemes which require about twenty generations to produce homozygosity, the approach being used with these swine involves selective breeding on the basis of histocompatibility typing of parents and offspring.

Four males and four females were purchased from commercial sources, and mating pairs were selected to be as varied as possible in order to assure the selection of different histocompatibility genes. Skin grafts were exchanged between the members of each pair and sera were obtained from the animals two weeks after the rejection of the grafts. The sera were tested for cytotoxic antibodies by lymphocytotoxic typing, anxpairs which produced strong cytotoxic antibodies were bred.

Offspring from each breeding were tested serologically to determine which histocompatibility antigens had been inherited from the parents, and offspring which could be shown to possess the same antigen combinations were selected for further breeding. This process has now been repeated for three consecutive generations. In addition, mixed lymphocyte culture (MLC) assays have been carried out in parallel to serologic testing of offspring.

Major Findings: 1) Naturally occurring anti-red blood cell antibodies of swine are cytotoxic to lymphocytes (unlike their human counterparts) and must be absorbed from sera with packed red cells before leukocyte typing is performed.

2) Skin grafts were rejected in 8-14 days in each pair tested, and the rejection was accompanied by the production of high-titered lymphocytotoxic antibodies in all but one pair.

3) Breeding was successful and typing of the second generation offspring showed four pairs which had inherited appropriate antigen combinations for further breeding.

4) Typing of the third generation offspring showed one possible breeding pair of animals homozygous for the same serologically determined transplantation antigens. While other homozygous animals were also obtained, none of these could be matched with an appropriate homozygous mate.

5) MCL assays of parents and offspring have shown a close correlation between this parameterof histocompatibility and the serologically determined transplantation antigens. Since this type of correlation has previously been observed in studies of man (HL-A), monkey (RhL-A), mouse (H-2), rat (AgB) and dog (DL-A), this finding indicates that we are selectively breeding for homozygosity at a MHC homologous to that of other mammalian species.

Significance to Biomedical Research and the Program of the Institute: One of the major problems in the study of cell surface antigens of human beings is lack of control of genetic constitution. The use of mice and rats as experimental models avoids this problem, but creates two new ones: 9) physiologically and anatomically these animals are often so different from human beings as to make comparisons and applications of findings difficult. 2) The size of these rodents makes it extremely difficult to obtain sufficient cells and tissues to permit quantitative chemical characterization of relevant cell surface antigens.

For both of these reasons it would be desirable to have animals of size comparable to human beings, whose genetic constitution with respect to histocompatibility could be controlled. The miniature pig, which attains an adult weight of about 200 lb. seemed ideal for this purpose, and to date the experimental breeding pattern outlined above appears to be working quite well.

Proposed Course of Project: The breeding plan and typing will be continued. Second generation animals will be re-bred in an attempt to obtain another homozygous breeding pair. At that point, lines will be established and separated, so that herds of any required size in each line can be produced.

Experiments envisioned once these lines are established include: 1) characterization of the immune response to transplantation within and between lines (in vitro and in vivo), 2) development of a transplantable tumor within one or more of the lines, and 3) isolation, purification and characterization of the major transplantation antigens of one or more of the lines.

Honors and Awards: None

Publications: None

Serial No. NCI-5024 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Antibodies to Cell-Surface Antigens of Leukemic Cells

Previous Serial Number: Same

Principal Investigator: Howard B. Dickler, M.D., David H. Sachs, M.D.

Other Investigators: William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives: 1) To develop antisera specific for tumor-related antigens on the surfaces of human chronic lymphocytic leukemia cells. 2) To determine the nature of the antigens detected. 3) To develop similar reagents for leukemic cells of mice to serve as a model for experiments using the anti-human leukemia reagents.

Methods Employed:

Human: Patients with a confirmed diagnosis of CLL and with both biologic parents alive were selected. Leukemia cells were purified from their peripheral blood and rabbits were immunized with these cells. The antisera are being tested for reactions with lymphoid cells and immunoglobulins of the patient, the patient's parents, other CLL patients, cultured lymphoblast lines, and normal controls. The intent will be to render the antisera specific for tumor-related antigens by absorption with the other cells and globulins. Tests for specificity of reactions will include cytotoxic screening, precipitin analyses, and immunofluorescent staining.

Mouse: Similar immunization and absorption procedures will be used on two or more recently derived lymphatic leukemias of mice which grow in ascites form. Normal lymphoid cells from syngeneic animals and from allogeneic animals will serve as the control cells for absorptions.

Major Findings: 1) Two appropriate patients have been selected and rabbit antisera have been raised against the leukemic cells of each. The antisera have high cytotoxic titers against normal and cultured lymphoid cells. Most of the antibody was directed against species-specific antigens. 2) One antiserum, after extensive absorption with cultured human lymphoblastoid cells and normal human PBL, had cytotoxic activity against PBL from several CLL patients but not against normal human PBL.

Significance to Biomedical Research and the Program of the Institute: 1) If detected, a tumor-related antigen common to all CLL cells would be of immediated use in diagnostic testing. 2) A tumor-specific reaction could have applications in the realm of immunotherapy. The mouse model would be of great importance at this stage. 3) If a true idiotype is detected in the surface immunoglobulin of the CLL cells, it would suggest the clonal nature of this disease. 4) The serologic reagents developed may provide an experimental approach to isolation and characterization of the relevant tumor-related antigens, which might in turn provide information on the etiology of the tumor.

Proposed Course of the Project: The availability of the donor CLL and his parents, as well as non-related CLL patients provide opportunities to control for inherited cell-surface antigens as well as determine whether the tumor-related antigens present are cross-reactive and/or individual specific. Large scale absorptions are being carried out with cultured human lymphoblastoid cells and normal human PBL in order to provide sufficient quantities of antiserum which lack antibodies against species specific antigens. The antiserum will then be absorbed with parental PBL or non-related CLL PBL at which point only tumor-related specificities should persist. Alternate absorption protocols will be used to develop possible serologic specificities toward "B" cell antigens, HL-A antigens, and possible idiotypic antigens of the cell-surface immunoglobulins.

Parallel immunizations and absorptions will be carried out for the mouse leukemia model. In vivo effects of the putative tumor-specific antibodies will be studied, and localization studies will be performed.

Honors and Awards: None

Publications: None

Serial No. NCI-5025(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Genetic Control of Antibody Responsiveness to Staphylococcal Nuclease

Previous Serial Number: NCI-5025

Principal Investigator: Gene M. Shearer, Ph.D. and David H. Sachs, M.D.

Other Investigators: Eugene C. Lozner, M.D.

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/2
Other	0

Project Description:

Objectives: The objective of this project was to investigate in detail the genetic regulation of immune response to nuclease in mice. This species was chosen since the genome of the mouse is better known than that of other rodents. Staphylococcal nuclease is advantageous as a model immunogen, since: (a) its amino acid sequence and three dimensional structure have been characterized; (b) the immunogen is a protein and appears to be more immunogenic than synthetic polypeptides; (c) a very sensitive enzymatic assay has been developed for detecting antibodies to nuclease; and (d) the molecule can be cleaved into a number of fragments which are capable of binding specific subpopulations of antibodies (Sachs, et al., J. Immunol. 109: 1300, 1972). It was also the purpose of this study to extend the studies in the rat to establish whether responsiveness to nuclease is linked to the major Ag-B histocompatibility region. Since genetic control of responsiveness to mitogens has been recently reported in rats (Newlin & Casser, J. Immunol 110: 622, 1973), possible genetic linkage between nuclease and mitogen response are also being tested.

Methods Employed: A number of inbred mouse strains as well as congenic resistant lines (differing only at H-2) were immunized with nuclease and their sera tested for anti-nuclease activity. Antibody activity was measured by determining the ability of given antiserum to inhibit nuclease hydrolysis of denatured DNA using a quantitative spectrophotometric technique. The number of anti-nuclease hemolytic plaque-forming cells detected in the spleens of immunized mice was determined using a modified Jerne assay. The antibody activity of sera specific for different determinants within the nuclease molecule was determined by hemolysis of tanned sheep erythrocytes coated with fragments of the enzyme. Since no congenic strains of rats are available, linkage studies were performed by following Ag-B histocompatibility typing and responsiveness to nuclease in successive generations of high and low responder rats. Similar mating studies have been performed in mice to verify linkage to H-2 or other linkage groups.

Major Findings: The major findings made thus far are:

1) Strain-dependent differences were detected in the antibody responses of a number of inbred mouse strains to nuclease. SJL/J mice were the highest responders to nuclease, whereas DBA/1 mice were the lowest responders. (DBA/1 x SJL/J)F_1 mice were also high responders.

2) The response potential appears to be H-2 linked to some extent. Thus, mice which elicited high antibody responses were H-$2^{a,s}$, intermediate responses H-$2^{d,k}$ and low responders H-$2^{b,q}$. Confirmation of H-2 linkage was obtained using the congenic lines B10 and B10.A. B10.A (H-2^a) mice were high responders, whereas B10 (H-2^b) mice were low responders. The immune response controlling responsiveness to nuclease in B10 mice was localized to the Ir region. More specifically, with the use of B10.A (2R), (4R), and (5R) recombinant strains, the gene(s) controlling responsiveness to nuclease was localized to the sight of Ir-1, in the same region as the gene controlling responsiveness to IgG myeloma.

3) Significant differences were detected in the number of IgM plaque-forming cells generated in high and low responder B10 mice, indicating that these H-2 linked genetic differences included differences in the IgM class of antibodies, in contrast to the findings of others using synthetic polypeptides (Grumet, J., Ext. Med. 135: 110, 1972).

4) The observations that a wide variation in the response to nuclease among different H-2^k strains of mice and that SJL/J are high responders whereas DBA/1 are low responders (similar to the non-H-2 linked responses of these two strains to (T,G)-Pro--L) raised the possibility that response potential to nuclease in these two strains might not be H-2 linked. However, H-2 typing of individual (DBA/1 x SJL)F_2 immunized mice revealed that both s/s and g/s mice were high responders whereas only the g/g progeny were low responders.

Among the various high and low responder strains, all appear to be synthesizing antibodies specific for a determinant contained in the 1-126 fragment of nuclease. Without exception, secondary immunization resulted in the production of antibodies specific for fragment 99-149 as well.

Significance to Biomedical Research and Program of the Institute: This program is relevant in that it examines the genetic regulation of immune responsiveness to a protein of well defined structure. Although synthetic polypeptides have provided important information concerning response genes, a study of such genes associated with responses to proteins can be more meaningful, since most naturally occurring antigens are complex and since protein antigens are generally better immunogens than are many of the simpler synthetic molecules. It remains to be established whether the immune response gene(s) regulating responses to nuclease (as well as those studied by others) will have any significant bearing on susceptibility to cancer or other diseases.

Proposed Course of Project: Attention will now be focused on stimulation of various immunological compartments with intact nuclease and nuclease fragments in order to define possible hapten and carrier functions of the original immunogen with respect to immune response gene expression, and to determine whether differences can be detected in antibody affinity between high and low responder strains.

Honors and Awards: None

Publications:

Lozner, E. C., Sachs, D. H., and Shearer, G. M.: Genetic control of the immunol. response to staphylococcal nuclease I: Ir Nase: Control of the antibody response to nuclease by the Ir region of the mouse H-2 complex. J. Exp. Med., in press.

Serial No. NCI-5026(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Genetic Control of Cellular Immunity to Staphylococcal Nuclease: Antigen-Induced Stimulation of Protein Synthesis

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: David H. Sachs, M.D., Eugene C. Lozner, M.D.

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	
Other:	1/4

Project Description:

Objectives: The objectives of this study were to establish whether the genetic control of antibody response to Staphylococcal nuclease could be extended to include differential stimulation of high and low responder lymphocytes in vitro for specific DNA or protein synthesis, and if so, to determine whether the incubation of monospecific alloantiserum with the lymphocytes would inhibit the DNA or protein synthesis.

Methods Employed: Lymph node cells from high and low responder mice which had been immunized with nuclease were cultured in microtiter plates with nuclease or nuclease fragments in a serum-free culture system. The cultures were pulse labeled for DNA or protein synthesis by incorporation of radioactive thymidine or leucine. Monospecific alloantiserum exhibiting specificity exclusive for H-2 were prepared using congenic B10 and B10.A mice. Other alloantiserum (specific for H-2 and other differences) was prepared in DBA/1 and SJL/J mice. The "blocking" of nuclease-induced lymphocyte stimulation is to be tested by incubating the cells with the antisera prior to culture with nuclease or nuclease fragments.

Major Findings: Staphylococcal nuclease specifically stimulated protein synthesis (but not DNA synthesis) in B10.A and SJL/J high responder mouse strains, without detectably elevating protein synthesis in B10 and DBA/1 low responder mice. This contrasts with the repeated observation that significant specific protein synthesis could not be obtained for synthetic polypeptide immunogens. Stimulation of protein synthesis by nuclease fragments 1-126 and 99-149 have given variable results. Lymph node cells fractionated over nylon wool columns have given interesting results. Both the adherent (B-cell) and non-adherent (T-cell) fractions gave greater stimulation of protein synthesis and nuclease than did unfractionated cells. Attempts to specifically inhibit nuclease-induced protein synthesis of high responder by monospecific H-2 alloantiserum was unsuccessful, since normal mouse serum inhibited the response to the same degree.

Significance to Biomedical Research and the Program of the Institute: These observations indicate that an immunological function other than antibody production is under genetic control in the mouse. This provides an opportunity to investigate the cellular aspects of immune response gene regulation at a level other than antibody production. Furthermore, it permits an analysis of the inhibition of lymphocyte stimulation by both restricted (H-2 differences only) and non-restricted (H-2 and other differences) alloantiserum in the elucidation of a possible Ir-gene product on cell surfaces.

Proposed Course of Project: Efforts will continue to be made to obtain protein stimulation with nuclease fragments and to correlate the genetic control of responsiveness to this immunogen with one of those fragments.

Honors and Awards: None

Publications:

Shearer, G. M., Garbarino, C. A., Sachs, D. H., Lozner, E. C., and Terry, W. D.: Genetic control of cellular immunity in mice to staphylococcal nuclease. *J. Exp. Med.*, submitted for publication.

Serial No. NCI-5027(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Identification of a Genetic Complex in the Mouse Other Than H-2 That Controls Multiple Immunological Functions and Antibody Response to More Than One Immunogen

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: David H. Sachs, M.D., Eugene C. Lozner, M.D., Edna Mozes, Ph.D., Gustavo Cudkowicz, M.D.

Cooperating Units: Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel; Department of Pathology, State University of New York Medical School, Buffalo, New York

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives: The H-2 region in the mouse and an analogous major histocompatibility region in the guinea pig have been shown to be genetic regions which control immunological responsiveness to a number of immunogens (McDevitt and Benacerraf, Advanc. Immunol. 11: 31, 1969). Nevertheless, other less obvious genetic complexes which regulate immunological functions for a number of immunogens could also exist. The Ir-3 gene is an example of a non-H-2 linked immuneresponse gene which controls responsiveness to the synthetic polypeptide (T,G)-Pro--L. SJL/J mice are the lowest responders to (T,G)-Pro--L, whereas DBA/1 mice are the lowest responders (Mozes, et al., J. Exp. Med. 130: 493, 1969). An identical pattern of responsiveness has also recently been observed for antibody responses to Staphylococcal nuclease (see separate report of Sachs and Shearer). The purpose of the present study was to establish whether a genetic region can be identified in the mouse other than H-2 which regulates multiple immunological function) and responsiveness to a variety of immunogens.

Methods Employed: The SJL/J and DBA/1 mouse strains as well as F_1, F_2

and backcrosses between these two strains are being studied with respect to their ability to respond to Staphylococcal nuclease, (T,G)-Pro--L, loop-Pro--L, and their ability to generate cytotoxic effector cells and anti-TNP plaque-forming cells to TNP-modified syngeneic lymphocytes, as well as their ability to reject hamster bone marrow cell grafts. The ability of individual F_2 and backcross animals to respond to the above mentioned antigens and immunological functions are being studied for possible association of responses as well as linkage to H-2 and allotype.

The animals are being sequentially immunized with nuclease and (T,G)-Pro--L and their sera assayed for anti-(T,G)-Pro--L activity by hemagglutination and anti-nuclease activity by a quantitative enzymatic assay (Sachs, et al. J. Immunol. 109, 1300, 1972). The experiments are being controlled for possible antigenic competition between these two immunogens. Expression of an Hh gene was tested by injecting hamster cells into irradiated F_1, F_2 and backcross animals whose response for the above-noted immunological parameters have been previously determined.

Major Findings: The major findings made thus far are:

SJL/J and DBA/1 mice exhibit differential responsiveness to nuclease in terms of antibody production and stimulation of protein synthesis. This response in these strains, however, was found to be H-2 linked. In contrast, the antibody responses to both the loop peptide of lysozyme and Pro--L are not H-2 linked, as is the ability to reject hamster marrow grafts. It remains to be established whether control of the cytotoxic and antibody responses of these strains are H-2 linked or not.

Significance to Biomedical Research and the Program of the Institute: If evidence can be obtained indicating the existence of a genetic region for expression of immune responsiveness other than H-2, then genetic regulation of immune responsiveness would be much more complex than is presently assumed, and another genetic complex of immune response will have been identified. Such an observation would expose an additional gene complex whose function for regulation of immune processes for both the normal and neoplastic tissue recognition could be pursued. It may be significant that the SJL/J mouse strain (which expresses immune gene functions as summarized above) is also a strain which develops viral-associated leukemia (Haran-Ghera & Kotler, Bibl. Haematol. 31, 70, 1968), and that the immune response potential of infected animals is reduced (Shearer, et al. J. Immunol. 110, 736, 1973).

Proposed Course of Project: Offspring of the F_1, F_2 and backcross mating are being individually studied with respect to their ability to respond to the immunological parameters outlined above. Should an association of two or more parameters be established, attempts will be made to link these immunological function to known murine genetic markers.

Honors and Awards: None

Publications:

Lozner, E. C., Sachs, D. H., and Shearer, G. M.: Genetic control of the immune response to staphylococcal nuclease I: Ir-nase: Control of the antibody response to nuclease by the Ir region of the mouse H-2 complex. J. Exp. Med., in press.

Serial No. NCI-5028 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Separation of Functionally Distinct Immunocompetent Cells by Fractionation over Histamine Columns

Previous Serial Number: Same

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: Elizabeth Simpson, Vet. M.B., Kenneth L. Melmon, M.D. Yacob Weinstein, Ph.D., Eugene C. Lozner

Cooperating Units: Division of Clinical Pharmacology, University of California Medical Center, San Francisco, Calif.

Man Years:

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives: It has been recently reported that fractionation of mouse spleen cells over columns of histamine-rabbit serum albumin-Sepharose results in a depletion of the number of anti-sheep erythrocyte plaque-forming cells (PFC) passing through the columns and in an elevation of the immune response potential of the same cell suspension when transferred into irradiated recipient mice (Shearer, et al., J. Exp. Med. 136: 1302, 1972). The objective of this study was to test the effects of such cell fractionation for other immunological functions, and to elucidate the cellular mechanisms involved.

Methods Employed: Histamine-rabbit serum albumin-Sepharose (HRSA-S) and rabbit serum albumin-Sepharose (RSA-S) beads were prepared by coupling histamine to RSA with ECDI, and then binding the proteins to CNBr-activated Sepharose 4B. Cells were incubated with the bead preparations (25 x 10^6 cells/ 0.6 ml of 25% W/v beads), and then non-adherent cells separated from the adherent population by passing the cell-bead mixture through a column containing a cotton plug. Cells were eluted from the HRSA-S and RSA-S columns by mechanical means. The various fractions of cells were tested for different immunological functions, e.g., in vitro and in vivo cell mediated cytotoxicity, grafts vs. host reaction, and the generation of PFC using in vivo cell transfers and in vitro Mishell-Dutton cultures.

Major Findings: The major findings can be summarized as follows: 1) Most of the cell mediated cytotoxic activity was found in the cells retained by the HRSA-S columns, when tested in vitro. In contrast, most of the cytotoxic precursor cell activity appeared to be in the cells that did not adhere to the HRSA-S columns when the cell mediated immunity was generated in irradiated allogeneic mice. 2) A similar discordance of results was observed between in vivo and in vitro experiments for humoral antibody responses to SRBC. In vivo cell transfers yielded elevated PFC responses in the cell population that did not adhere to the histamine beads, whereas no effect of the column could be detected when the cells were cultured in vitro. 3) No effect of this type of cell separation was observed on the graft versus host response tested in vivo. 4) Soluble histamine introduced into the cultures reduced cell mediated cytotoxicitiy during the early phase of immunization (days 1 and 2) but not later (days 3 and 4). 5) The effect of histamine-column separation on the enhancement of antibody response potential was found to be due to T-cells and not B-cells. 6) The possibility that the immunobiological effects of the histamine columns was due to fractionation of cells on a charge basis was ruled out since poly-L-lysine columns (similar in charge to histamine) had no effect on either the antibody or cytotoxic responses.

Significance to Biomedical Research and the Program of the Institute: These results raise the possibility that certain populations of lymphocytes may express surface receptors for histamine. The fractionation procedures can be utilized for separation of cells with different immunological functions. The differential effects of testing the separated cells in vitro and in vivo raises the possibility that radio resistant cells in the host animals may modify immune responses and/or that in vitro culture systems may permit the survival of selected cell populations. This study suggests that receptors for endogenous hormones may be expressed on lymphoid cells and they may be significant in regulating immune processes.

Proposed Course of Project: The in vivo versus in vitro results will be pursued to determine if the differences observed could be due to differences in effective antigen doses or cell concentration effects. Attempts will be made to alter the in vitro cell mediated and humoral immune responses with soluble histamine and other beta adenergic hormones and to block their effects by specific hormone antonists.

Honors and Awards: None

Publications:

Gene M. Shearer, Yacob Weinstein, and Kenneth L. Melmon: Enhancement of immune response potential of mouse lymphoid cell fractionated over histamine columns. J. Immunol., submitted for publication.

Shearer, G. M., Simpson, E., Lozner, E. C., Weinstein, Y., and Melmon, K.L.: Modification of cell-mediated immunity by lymphocyte fractionation over columns of insolubilized histamine. Eur. J. Immunol., submitted for publication.

Serial No. NCI-5029(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Preparation of Tumor and Tissue Specific Antisera From Human Cancers

Previous Serial Number: Same

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: None

Cooperating Units: Richard L. Edelson, M.D., Marvin A. Lutzner, M.D., Dermatology Branch, NCI; Philip S. Schein, M.D., Medicine Branch, NCI

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: To prepare serologic reagents with specificity for human tumor antigens or tissue specific antigens present on tumors for the purpose of diagnostic and prognostic studies, for identification of tumor cells in pathological specimens and in mixed culture.

Methods Employed: Standard tissue culture techniques, immunization of rabbits and mice, absorption with cells, tissues, or serum as indicated, cytotoxic and immunofluorescent assays. Lymphoblast lines from some patients are being established in order to make available genetically identical absorbing cells.

Major Findings: Antisera were prepared against cultured melanoma cell line cells. These sera were absorbed with cultured human lymphoblast cell lines and tested for complement dependent cytotoxicity against explanted melanoma cells, melanoma cell line cells, nevus cells, fibroblasts, cultured breast and ovarian tumor cells, and peripheral lymphocytes. A new ^{51}Cr release microassay method permitted evaluation of reagents using only minute quantities of antisera and cells.

The absorbed anti-melanoma serum (AMS) killed all tested cell line cells, as well as all freshly explanted melanoma cells and normal nevus cells. In contrast, breast and ovarian cancer cells, skin fibroblasts, and cultured ovarian and breast carcinoma cells were not killed and could not absorb activity out of the antiserum. All tested melanoma cell lines fully removed anti-melanoma activity. Some specificity was also demonstrated for ovarian carcinoma cells and for breast tumor cells using a similar approach.

Significance to Biomedical Research and the Program of the Institute: Reagents for tissues or tumor specific antigens will be of great value in diagnosis of tumors if they secrete antigens into blood or urine and will aid pathological diagnosis of surgical specimens.

Proposed Course: Attempts to prepare sera specific for antigens on melanoma, breast carcinoma, ovarian carcinoma and lymphatic malignancies will be continued.

Honors and Awards: None

Publications:

Smith, R. W.: ^{51}Cr release microassays for histocompatibility antigens on normal and malignant cells. *Transplantation* 16:246, 1973.

Serial No. NCI-5031(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Human Lymphocyte Markers in Basic Research and in Lymphoid Diseases

Previous Serial Number: Same

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: None

Cooperating Units: R. Michael Blaese, M.D., Metabolism Branch, NCI; William Lewis, M.D., Richard L. Edelson, M.D., Dermatology Branch, NCI; Paul Pattengale, M.D., Paul Gerber, Ph.D., Bureau of Biologics; Brigid G. Leventhal, M.D., Hematology and Supportive Care Branch, NCI; Ivor Royston, M.D., Bureau of Biologics, James Woody, M.D., Naval Medical Research Institute

Man Years:

Total:	0
Professional:	1/2
Other:	1/2

Project Description:

Objectives: The objectives of this project include various goals in basic and clinical immunology: 1) to develop and to standardize serologic reagents for reliable identification and selective elimination of human T and B lymphocyte. 2) the comparison of sensitivity and reliability of five different markers of human T or B lymphocytes, since these markers are being used to determine the type of lymphocytes participating in different types of cellular immune reactions *in vitro* and *in vivo*. 3) to study the cells responding to mitogens and to viruses. 4) to determine the type of lymphocytes participating in cellular immune reactions *in vivo* and *in vitro*. 5) to study lymphocyte type and function in various immunodeficiency syndromes as well as in malignant diseases such as lymphoma and lymphocytic leukemia.

Methods Employed: Freshly explanted and cultured cells are being tested simultaneously for T and B lymphocyte markers. Likewise normal cord blood lymphocytes are being transformed by E-B virus in an effort to determine which lymphoid populations are susceptible to virus-induced transformation. In parallel studies, mononucleosis and Burkitt's lymphoma cell lines are being characterized. Malignant and normal lymphocytes from leukemia patients are being established in culture, as are cells from patients with agammaglobulinemia and other immunological diseases. Lymphoid infiltrates in tumors are being examined for evidence of cellular immunity. Antilymphocyte sera prepared in several species with a variety of immunogens was absorbed to render it specific for either T or B lymphocytes. Complement dependent cytotoxicity, lymphocyte dependent cytotoxicity, and fluorescent antiglobulin techniques were used to evaluate specificity.

Tests for surface Ig, the SRBC, Fc, and C'3 receptors were performed in parallel with the above assays.

Major Findings: 1) The newly developed HBLA and HTLA reagents have been shown to be specific markers for B and T lymphocytes respectively, and have been shown to be still present under conditions such as malignancy, immunodeficiency disease, or long-term culture where other markers are lost. The normal human lymphoid system can be divided into two mutually exclusive antigenic compartments roughly corresponding to the T and B compartments of the mouse. These compartments can be defined by the presence of either Human Thymic Lymphocyte Antigens (HTLA) or Human Bone Marrow Lymphocyte Antigens (HBLA). The remaining markers overlap into both of the above categories. Thus, some T cells have C'3 and Fc receptors, while some B cells at times express SRBC receptors. Anti HTLA antibody content of ALS is related to immunosuppressiveness *in vivo*.

2) Cellular immunity *in vitro* against some tumors is a T cell phenomenon.

3) Lymphocyte dependent antibody mediated cellular immune reactions are due to B cells.

4) Acute lymphocytic leukemia is a T cell disease, although the sheep red cell rosette assay may be negative.

5) Patients with the Wiskott-Aldrich Syndrome have normal numbers of T cells in the peripheral blood, although the number of sheep red cell rosette forming cells may be very low.

6) The E-B virus appears to preferentially transform B cells.

7) T and B lymphocyte population can be separated by newly developed techniques or modification of existing techniques.

8) T and B lymphocytes are patent stimulation of MLC reactions.

Significance to Biomedical Research and the Program of the Institute: Better means of identifying and separating human lymphocyte subpopulations now permits experiments designed to elucidate immune mechanisms, subclassify clinical disease such as ALL, and provide new information on the pathogenesis of lymphoid diseases. The HTLA and HBLA reagents permit precise identification of T and B human lymphocytes and will facilitate a wide variety of basic and clinical investigations and more sophisticated monitoring of human immune responses. Measurement of HTLA will permit *in vitro* standardization of the immunosuppressive potency of antilymphocyte serum used in clinical transplantation.

Proposed Course of Project: As I am leaving the NIH June 30, 1974, this project will be discontinued here.

Honors and Awards: None

Publications:

Smith, R. W., Blaese, R. M., Hathcock, K. S., Buell, D. N., Edelson, R. L., and Lutzner, M. A.: T and B lymphocyte markers in lymphoid cell research and in human diseases. In (Lindahl-Kiessling, K and Osaba, D. eds) *Proceedings of the Eighth Leukocyte Culture Conference - Lymphocyte Recognition and Effector Mechanisms*. Academic Press, New York, 1974, pp. 127-132.

Pattengale, P. K., Smith, R. W. and Gerber, P.: Selective transformation of B lymphocytes by EB virus. *Lancet* ii:93, 1973.

Pattengale, P. K., Smith, R. W. and Gerber, P.: B cell characteristics of human peripheral blood lymphocytes transformed by Epstein-Barr virus. *J. Nat. Cancer Inst.*, in press.

Edelson, R. L., Kirkpatrick, C. M., Shevach, E. M., Schein, P. S., Smith, R. W., Green, I. and Lutzner, M. A.: Preferential cutaneous infiltration by neoplastic thymus derived lymphocytes: Morphologic and functional studies. *Ann. Intern. Med.*, in press.

Smith, R. W., and Woody, J. N.: The immunosuppressive potency of antilymphocyte serum is related to activity against Human Thymic Lymphocyte Specific Antigens (HTLA). *Transplantation*, in press.

Serial No. NCI-5033 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunotherapy of Human Cancer

Previous Serial Number: Same

Principal Investigator: William D. Terry, M.D.

Other Investigators: Richard Fisher, M.D.

Cooperating Units: William Levis, M.D., Dermatology Branch, NCI; Paul Chretien, M.D., Surgery Branch, NCI; Vincent DeVita, M.D., Medicine Branch, NCI; Ayub Ommaya, M.D., Surgical Neurology Branch, NINDS

Man Years:

Total:	1 1/2
Professional:	1 1/2
Other:	1/2

Project Description:

Objectives: This project is designed to study various approaches to the immunotherapy of human malignancies.

Methods Employed: Patients will be evaluated for immunologic status and treated with a variety of immunotherapeutic maneuvers. They will be monitored for changes in general immune responsiveness, as well as for specific responsiveness to tumor antigens.

Major Findings: (1) A study of immunotherapy of human melanoma was initiated in collaboration with the Medicine and Surgery Branches. Allogeneic melanoma cells in long term culture were obtained from Dr. Fogh (Sloan Kettering Institute). Cells were converted to growth in human serum. Tests performed by Dr. Ingegard Hellstrom indicated that these cells contained a cell surface antigen recognized by lymphoid cells of patients with melanoma but not by lymphoid cells of patients with other malignancies, as determined by the microcytotoxicity test used in Dr. Hellstrom's laboratory. The cell line (RPMI-8072) was grown up in large quantities (Contract N01-CB-02207), treated with neuraminidase and mitomycin and frozen in vials. Vaccine was tested for sterility and pyrogenicity.

Two studies are currently in progress using this vaccine.

A. Patients with stage III disease and with observable lesions are randomized into two groups. One receives methyl-CCNU, the other is immunized with vaccine and BCG. Vaccine is given as an intradermal injection with BCG applied to the skin over this site with a Heaf gun. Results are summarized on the following table:

	Chemotherapy	Immunotherapy
Total	8	10
Partial regression	0	1
Stable disease	1	1
Progressive disease	6 (2)*	5 (3)*
Too early to evaluate	1	3

( )* =number who have expired.

This study was designed basically to be a toxicity study, and has shown that the vaccine and BCG can be given without acute toxic effects. Evaluation of the effects of the immunization on the immune system is in progress.

B. Patients with stage II melanoma with no evidence of residual disease are randomized into 3 groups. One is treated with methyl-CCNU, one with vaccine and BCG, and one is not treated at all (present acceptable treatment for stage II melanoma). This study was started very recently and few patients have been admitted. They are summarized on the following table:

	Chemotherapy	Immunotherapy	Control
Total patients	2	3	4
Disease free	1	1	3
Too early to evaluate	0	2	1
Recurrent disease	1	0	0

This study is in its very early stages and it is estimated that some 200 patients will have to be treated and followed to permit statistical evaluation of effects.

(2) Studies of patients with basal cell carcinoma have continued, and the results of the first phase of the study published. General conclusions are that DNCB can cause regression of some, but not all, basal cell carcinomas and confirms the work of Edmund Klein.

(3) A study has been initiated with the Surgical Neurology Branch in which patients with glioblastoma have their tumors removed and a cyst-like prosthesis inserted in the tumor bed. Chemotherapy is administered through this prosthesis. In some of the patients, PPD is also administered after the patients have been sensitized with BCG.

Significance to Biomedical Research and the Program of the Institute: Immunotherapy studies will explore the clinical effectiveness of manipulating the immune system in patients with cancer, and will also provide new information about the biology of the tumor-host relationship.

Proposed Course of Project: (1) Stage III melanoma studies will be modified to test combinations of chemotherapy and immunotherapy as well as escalating doses of immunotherapy. Stage II melanoma studies will be modified to test combinations of chemotherapy and immunotherapy, as well as escalating doses of immunotherapy.

Stage II melanoma studies will be modified to include most stage II patients. At the present time only those with the worst prognosis are included.

(2) The usefulness of immunotherapy of skin cancer will continue to be explored.

(3) The study of glioblastoma will continue.

Honors and Awards: None

Publications:

Levis, W. R., Kraemer, K. H., Klingler, W. D., Peck, G. L. and Terry, W. D.: Topical immunotherapy of basal cell carcinomas with dinitrochlorobenzene. Cancer Research 33: 3036-3042, 1973.

Serial No. NCI-5034(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization and Isolation of Human Lymphocytic Subpopulations

Previous Serial Number: None

Principal Investigator: Howard B. Dickler, M.D.

Other Investigators: John R. Wunderlich, M.D., Ronald H. Schwartz, M.D., Barry S. Handwerger, M.D., Richard J. Hodes, M.D., William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	3/4
Other:	1/4

Project Description:

Objectives: To characterize and isolate human lymphocyte subpopulations and evaluate the function of such populations in the immune response in both normals and disease states.

Methods Employed: Lymphocytes from normal humans and patients with various diseases are isolated and evaluated for surface Ig and the receptor for complexed antibody (primarily B cell markers) and the formation of spontaneous rosettes with SRBC (a T cell marker). Lymphocyte subpopulations are isolated using nylon columns, a fluorescent-activated cell sorter and density flotation centrifugation procedures.

Major Findings: 1) Peripheral blood lymphocytes from normal humans were found to consist of 4 subpopulations: B cells, T cells, cells bearing both B and T cell markers (double marker cells), and cells bearing no markers (null cells). 2) Evaluation of lymphocyte surface markers has allowed separation of patients with variable immunodeficiency and panhypogammaglobulinemia into distinct groups which appear to differ in the nature of their fundamental defect. 3) Patients with chronic lymphocytic leukemia could be separated into T and B cell types, the latter even when surface Ig was not easily detected.

4) Significant enrichement of both B and T cell populations has been obtained using the fluorescent activated cell sorter, nylon columns, or density flotation centrifugation.

Significance to Biomedical Research and the Program of the Institute: Characterization and isolation of lymphocyte subpopulations is of fundamental importance for an understanding of the nature of the immune response.

Characterization of lymphocyte surface markers has provided further insight into the nature of the basic defects in some patients with immunodeficiency.

Proposed Course of Project: Isolated subpopulations of lymphoid cells and clonal populations from disease states will be used to develop reagents specific for each subpopulation. Isolated subpopulations will be evaluated for functional capabilities in the immune response, both alone and in combination.

Honors and Awards: None

Publications:

Dickler, H. B., et al.: Lymphocyte binding of aggregated IgG and surface Ig staining in chronic lymphocytic leukemia. Clin. Exp. Immunol. 14: 97, 1973

Dickler, H. B., Adkinson, N. F. and Terry, W. D.: Evidence for individual human peripheral blood lymphocytes bearing both B and T cell markers. Nature 247: 213, 1974

Dickler, H. B., Adkinson, N. F., Fisher, R. I., and Terry, W. D.: Lymphocytes in patients with variable immunodeficiency and panhypogammaglobulinemia: Evaluation of B and T cell surface markers and a proposed classification. J. Clin. Invest., in press.

Serial No. NCI-5035 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunochemical, Genetic, and Functional Analysis of the Lymphocyte Receptor for Complexed Immunoglobulin

Previous Serial Number: None

Principal Investigator: Howard B. Dickler, M.D.

Other Investigators: David H. Sachs, M.D., John R. Wunderlich, M. D., Pierre A. Henkart, Ph.D.

Cooperating Units: None

Man Years:

Total: 1 3/4
Professional: 1
Other: 3/4

Project Description:

Objectives: (1) To isolate and immunochemically characterize the lymphocyte receptor for complexed antibody; (2) to evaluate the genotypic and phenotypic expression of the receptor; (3) to evaluate the role of this receptor in the immune response.

Methods Employed: Heat-aggregated immunoglobulin and/or antigen-antibody complexes are fluorochrome or radioactive isotope labeled, and then allowed to interact with isolated lymphocytes from both man and animals. Binding is assayed by U.V. and phase microscopy or by isotope counting. The interaction is studied by manipulation of the conditions of interaction, and by immunologic and chemical modifications of both lymphocytes and complexes. Genetic studies are carried out utilizing inbred, congenic, and/or recombinant mouse strains; or patients with immunologic abnormalities. Lymphocyte membrane components are solubilized using non-ionic detergents, and components which bind to complexes can be further analyzed by electrophoretic and chromatographic procedures. Functionally, antibody-dependent, cell-mediated cytolysis has been utilized.

Major Findings: The receptor for complexed immunoglobulin is not dependent on complement, temperature, pH or divalent cations. It is distinct from surface Ig and is a trypsin-resistant protein or glycoprotein. The receptor is specific for the Fc portion of complexed Ig and the site on the Fc involves a disulfide bond(s). The receptor is necessary for antibody-dependent cell-mediated cytolysis.

Significance to Biomedical Research and the Program of the Institute: The receptor for complexed antibody has been shown to play a key role in antibody-dependent cell-mediated cytotoxicity. This immunologic mechanism may play an important part in host response to tumors. This receptor may also be important in regulation of the immune response and/or collaboration between immunocompetent cells.

Proposed Course of Project: Attempts will be made to isolate and further characterize the receptor for complexed Ig. The phenotypic and genotypic expression of the receptor will be analyzed. The role(s) of this receptor in the immune response will be further studied.

Honors and Awards: None

Publications:

Dickler, H. B.: Studies of the human lymphocyte receptor for heat-aggregated or antigen-complexed immunoglobulin. Manuscript in preparation.

Serial No. NCI-5036 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Relationship Between Ir Genes and Immunoglobulin Variable Region Genes

Previous Serial Number: None

Principal Investigator: David H. Sachs, M.D. and C. Garrison Fathman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives: The antibody response to staphylococcal nuclease (NASE) is under genetic control by gene(s) located in the Ir region of the mouse major histocompatibility complex (H-2). Since the antibodies produced to certain regions of this antigen have been previously shown to demonstrate restricted heterogeneity, we wish to determine whether or not the relevant Ir gene determines which variable region immunglobulin products are produced. If the Ir gene is found to exert quantitative but not qualitative control over idiotypic antibody production, then a serach for a distinct "T cell receptor" for nuclease (i.e., other than known IgV regions) must be anticipated.

Methods Employed: (1) Antibodies to NASE are being prepared in groups of mice of a variety of strains (both high and low responders) differing in H-2 type or allotype or both. These will be purified by affinity chromatography. Inbred Lewis rats will be immunized with the purified anti-NASE antibodies from several of the high-responder strains.

Immunoabsorptions with normal immunoglobulins from the same strain will be used in order to determine whether or not antibodies reactive with the variable region (i.e., anti-idiotypic antibodies) have been produced.
(2) Anti-idiotypic reactions will be quantitated by the inhibition of antibody-mediated enzyme inhibition. Such reactivities will then be screened against anti-NASE antibody populations from the other strains of mice in order to determine the possible genetic linkage of idiotype to allotype and/or H-2 type.

(3) Attempts will be made to block functional recognition of NASE by lymphocytes using appropriate anti-idiotypes. Depending on the genetic linkage that is found, emphasis will be placed on distinguishing T cell from B cell specificity.

(4) The possible expression of idiotypic determinants on specific cell surfaces will be examined using fluoresceinated anti-idiotype and the Fluorescence-Activated Cell Sorter (FACS).

Major Findings: A/Jax anti-NASE antibodies have been successfully purified and used for immunization of rats. Preliminary inhibition assays indicate that the rat antiserum produced detects an idiotypic determinant on the immunogen.

Significance to Biomedical Research and the Program of the Institute: The development of an anti-idiotypic reagent directed toward antibodies under the control of an H-2 linked Ir gene may provide insight to the mechanism of Ir gene function. It may indicate whether the specificity of T cell recognition of antigens depends on similar variable region gene products to those responsible for B cell or antibody specificity. It may also lead to possible methods of controlling Ir gene expression.

Proposed Course of Project: Additional anti-idiotypic antisera will be raised against the anti-NASE antibodies of other strains of mice. The reactions of each will be characterized by inhibition of binding assays or by radioimmunoassays if necessary. Screening studies will then be performed in order to determine the genetic linkage of the idiotypic specificities distinguished.

If linkage of idiotype expression with H-2 type is established, we will move directly to studies of cell surface receptors on T cells and to manipulations of responsiveness by means of pretreatment with the anti-idiotypic reagents.

If linkage to allotype is established, this relationship will be fully characterized, since previous studies of such linkage have not employed antigens under Ir control. An attempt will then be made to immunize with immune T cells rather than antibody in order to determine whether another "idiotype" can be distinguished for the T cell receptor.

Honors and Awards: None

Publications: None

Serial No. NCI-5037(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cell Mediated Immunity to Carboxy Puridine Disulfide-Modified Syngeneic Lymphocyte Proteins: Linkage of Responsiveness to the Murine Major Histocompatibility Complex

Previous Serial Number: None

Principal Investigators: Gene M. Shearer, Ph.D. and Pierre A. Henkart, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives: As described in an accompanying report, it has been possible to generate cell mediated immunity to TNP-modified syngeneic lymphoid cell proteins. The ability to respond was found to be linked to the H-2 complex, as were the specificity restrictions between immunogen and target. The objective of the study outlined here was to determine whether modification of cell surface proteins with protein-binding reagents other than trinitrobenzene sulfonic acid would result in changes that would create immunogenic determinants recognized as foreign by syngeneic lymphocytes, and to investigate the genetic and immunologic parameters described in the accompanying report.

Methods Employed: Lymphoid cells from a number of inbred and congenic resistant mouse strains were heavily modified by treatment with carboxy pyridine disulfide (CPDS). These modified cells were used as immunogens and radioactively labelled target cells for immodified syngeneic and allogeneic responding lymphocytes, in primary in vitro sensitization for CML reactivity. Effector cells from the CML sensitization were assayed for cytotoxicity on syngeneic ^{51}Cr-labelled targets.

Major Findings: H-2 linked genetic control of responsiveness to CPDS-modified syngeneic lymphocytes has been found. All B10 congenic lines were non-responders to CPDS except B10.D2, which was the only responder strain found to date. This contrasts with TNP modification in which B10 was only non-responder (see accompanying report).

Significance to Biomedical Research and Program of the Institute: The same possible points of relevance mentioned in the TNP report apply here as well.

Proposed Course of Project: The same general course of study outlined in the TNP report will be pursued here. Furthermore, since B10.D2 mice are responders to both TNP- and CPDS- modified syngeneic lymphocytes, interesting competition experiments at both the molecular and cellular levels can be performed to determine if the same protein(s) is being modified by both reagents and if any immunological cross-reactivity can be demonstrated.

Honors and Awards: None

Publications: None

Serial No. NCI-5038(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cell Mediated Immunity to Trinitrophenol-Modified Syngeneic Lymphocyte Proteins: Linkage of Responsiveness and Specificity of Reactivity to the Murine Major Histocompatibility Complex

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: Eugene C. Lozner, M.D., David H. Sachs, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives: The purpose of this project was to investigate *in vitro* induction of cell mediated immunity, i.e., cell mediated lympholysis (CML) and mixed lymphocyte reactivity (MLR) of murine lymphocytes to syngeneic and allogeneic lymphoid cells (normal and neoplastic) modified with trinitrophenol (TNP). This study was designed to establish: a) whether the immune reactivity obtained was specific for the haptenic moiety (TNP) or whether it involved reactivity to some TNP-modified cell surface protein(s); b) whether response potential in this model was associated with an immune response (Ir) gene, and if so to determine the linkage pattern; c) how specific the reactivity is *vis a vis* the genetic and cellular components of the induction and effector phases; d) if the immune reaction is composed exclusively of T cells or if B cells are also required; and e) whether antibodies specific for TNP are generated in addition to the cell mediated response during the course of immunization.

Methods Employed: Lymphoid cells from a number of inbred and congenic resistant mouse strains were heavily TNP-lated by treatment with trinitrobenzene sulfonic acid. These modified cells as well as lymphoma cells were used as immunogens and as radioactively-labelled cytotoxic target cells for unmodified syngeneic and allogeneic responding lymphocytes in primary in vitro sensitization for either CML or MLR reactivity. Effector cells from the CML sensitization were assayed for cytotoxicity on the appropriate (identical and nonidentical to genotype of immunizing cell type) ^{51}Cr-labelled targets and tested for anti-TNP antibody by a TNP-coated sheep erythrocyte plaque assay.

Major Findings: The major findings made thus far are:

1) Cell mediated cytotoxicity to TNP-modified syngeneic spleen and thymocytes but not to tumor cells was obtained. By assaying the sensitized lymphocytes with TNP-modified cells from an unrelated strain, exclusive reactivity to TNP was ruled out. The response appeared to be directed either to a modified cellular protein or to the modified protein plus the TNP moiety.

2) Cross reactivity between the modified immunogen and the modified target could be obtained when the immunogen and target shared a K-end or D-end H-2 specificity. Cross reactivity was about twice as great for a common K-end than for a common D-end region.

3) The ability to respond to TNP-modified syngeneic lymphocytes is controlled by an H-2 linked Ir gene in the B10 congenic mouse lines. Of the strains thusfar tested, only H-2^b mice do not respond to TNP-modified spleen cells. Ability to respond is dominant. Inability to respond in the B10 strain has been found to be due to the failure of this strains lymphocytes to form an immunogen which recognized by responder B10.A mice. TNP-modified B10 lymphocytes do form an immunogen, however, which cross reacts with and is recognized by SJL/J lymphoid cells.

4) Another mouse strain unrelated to the B10 congenic lines studied is SJL/J. This strain responds to TNP-modified syngeneic spleen cells, but differs from those described above in that there is cross reactivity with TNP-modified B10 and B10.A but not B10.D2 lymphocytes.

5) Some limited success has been obtained with MLR using TNP-modified syngeneic spleen cells as the stimulating population.

6) TNP-modified CML to syngeneic spleen cells appears to be age-dependent, since the use of mice older than 2 months of age has been consistently unsuccessful. The effector phase was shown to be sensitive to rabbit anti-mouse brain serum and is therefore compatible with it being a T cell.

7) Although the CML appears to involve a modified cell protein, concominant antibody production to TNP can be obtained. A higher plaque response was observed when the responding cells were sensitized with TNP-modified syngeneic cells than with TNP-modified allogeneic cells.

8) Modification of syngeneic thymocytes also resulted in CML. However, sensitization with thymocytes was detectable only with TNP-modified thymocytes, whereas sensitization with modified spleen cells could be assayed on TNP-modified spleen, lymphoma or thymus cells. This observation suggests that for

immunization, thymocytes may be modified in a different way than spleen cells.

Significance to Biomedical Research and Program of the Institute: This project is of basic immunological interest in that it describes a new murine major histocompatibility-linked immunological phenomenon. The fact that the phenomenon itself involves immunological reactivity to modified self lymphocyte surface proteins raises the possibility that this is a type of autoimmunity which is H-2 linked. This observation could provide an initial model for investigating the immunological effects of viral-modified lymphocyte surface proteins. Immune response gene products have been postulated to exist. Since the Ir defect of this system resides in failure of a cell surface protein to be modified so as to be immunogenic to syngeneic lymphocytes, and since the gene(s) controlling this map in the Ir region of H-2, the protein(s) modified are possible candidates for the elusive Ir gene product.

Proposed Course of Project: Additional strains of mice will be screened for genetically determined responsiveness to TNP-modified self-proteins, particularly those strains which are known to have autoimmune diseases such as NZB and NZW, as well as strains shown to exhibit viral-associated leukemogensis such as SJL/J, both prior and subsequent to the onset of disease. Studies will be performed to determine whether the protein(s) modified by TNP are immune response gene products and/or immune response-associated antigens. Attempts will be made to solubilize, isolate, and characterize the modified cell surface proteins. Finally, once the model has been genetically, chemically, and immunologically characterized, attempts will be made to create "foreign immunogens" using syngeneic cells by modification with viruses. Studies are also in progress to determine whether a similar immunological model can be demonstrated using human leukcocytes from normal and autoimmune individuals, and to determine whether any correlation exists between the response potential of individuals and HL-A type.

Honors and Awards: None

Publications:

Shearer, G. M.: Cell mediated cytotoxicity to TNP-modified syngeneic lymphocyte proteins. Eur. J. Immunol., submitted for publication.

Serial No. NCI-5039(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Fractionation of Lymphoid Cells Using Nylon Wool Columns

Previous Serial Number: None

Principal Investigator: Ronald H. Schwartz, M.D., Ph.D.

Other Investigators: Barry S. Handwerger, M.D., Richard J. Hodes, M.D., William D. Terry, M.D.

Cooperating Units: Laboratory of Biochemistry, NCI, E. Brad Thompson; Clinical Endocrinology Branch, NIAMDD, A. Raffaele Bianco

Man Years:

Total: 1
Professional: 1
Other:

Project Description:

Objectives: 1) To identify subpopulations of lymphocytes participating in various immunological reactions in order to gain an understanding of the physiology of the immune system. 2) To investigate the nature of hormonal influences on the immune system by identifying their sites of action.

Methods Employed: During the past year our laboratory has made a significant advance in the separation of mouse lymphoid cells. We have helped develop a simple, rapid, inexpensive and efficient technique for separating large numbers of mouse spleen and lymph node cells into two populations, one enriched for thymus-derived or T lymphocytes and the other enriched for bone marrow-derived or B lymphocytes. The procedure is an extension of a technique first described by Julius, Simpson and Herzenberg (Eur. J. Immunol. 3: 645, 1973). It involves passage of cells over nylon wool columns at 37°C in the presence of Eagle's medium containing 5% heat-inactivated fetal calf serum. The B cells preferentially adhere to the columns, whereas the T cells pass through. After washing the T cells through the columns, the B cells are removed by mechanical agitation. Preliminary experiments indicate that this technique can be extended to other species including humans.

Major Findings: This technique is now being utilized to explore the role of T and B lymphocytes in various immunological reactions. The nature of the stimulating and responding cells in the mixed leukocyte interaction has been investigated by fractionating the cells of one mouse strain on nylon wool and then incubating the separated B and T cells with unfractionated cells from another mouse strain. Preliminary experiments suggest that the B cell enriched population is a better stimulator whereas the T cell enriched population is a better responder. This has proven to be the case in both congenic and noncongenic combinations of mice. These experiments, furthermore, suggest that Ir-associated alloantigens may act as targets for MLC stimulation.

A similar approach has been used in determining the nature of the stimulator and responder cell types in an allogenic in vitro sensitization and cell-mediated cytotoxicity assay system. In this system, however, the data have been more complex. It appears that unfractionated cells are the best stimulating population. This suggests that some cell which is necessary for stimulation is not recovered from the columns. In the case of the responder cell, the best population appears to be a mixture of the two separated populations. Moreover, the response of the mixture is greater than the sum of the responses of its component parts. This synergistic response implies that more than one cell type is participating in the sensitization-cytotoxicity response. Both the B cell-enriched and T cell-enriched populations demonstrate excellent in vitro sensitization and cytotoxicity. Experiments are now in progress to determine the exact nature of the responding cell in both fractionated populations and the nature of the cells responsible for the observed synergy.

Finally, in recent studies we have begun to compare control lymphoid tissue (thymocytes and cortisone resistant thymocytes [CRTs]) with peripheral lymphoid tissue (spleen and lymph node cells) as responders and stimulators in both the MLI and cytotoxic assays. So far it is clear that CRT's are the best sensitizers we have encountered in either assay system.

In an entirely different area, the columns are being put to use in analyzing the type of lymphoid cells which have receptors for various hormones. In collaborative work with Dr. E. B. Thompson we have examined the glucocorticoid receptor in mouse lymphoid tissues. Preliminary results indicate that thymocytes have a large amount of the binding protein whereas peripheral lymphoid tissues have lower levels, spleen possibly being greater than mesenteric lymph node. Nylon column fractionation of spleen and lymph node cells is presently underway to see if the small but significant amount of binding protein in the peripheral tissues can be localized to B or T lymphocytes. An entirely different result has emerged from our studies on the insulin receptor (done in collaboration with Dr. A. Raffaele Bianco). In the mouse neither the T-enriched populations of mouse spleen fractionated on nylon wool columns showed much binding of ^{125}I-labelled insulin. Similarly, purified peripheral blood lymphocytes from humans bound very little insulin. However, populations of human PBL containing monocytes did bind insulin. In a series of depletion experiments using nylon, glass wool and sephadex G10 we were able to show that insulin binding correlated with the number of phagocytic cells in the preparation and not with the number of lymphocytes staining with fluorescein-conjugated anti-human immunoglobulin (presumptive B lymphocytes). Current studies are aimed at directly visualizing the binding of ^{125}I insulin to monocytes using autoradiography.

Significance to Biomedical Research and the Program of the Institute: Before the immune system can be optimally manipulated for clinical purposes the nature of the various cells participating in its functions must be unraveled. The separating technique we have described should be useful for this purpose. Studies of *in vitro* mixed lymphocyte interactions and direct cell-mediated cytotoxicity serve as excellent models for transplantation rejection and possibly also for tumor immunity. Thus, understanding the types of immune cells involved in these reactions should ultimately prove of benefit in clinical medicine. In addition the ability of this technique to handle large numbers of cells in a short period of times makes it useful for carrying out biochemical studies on lymphocyte subpopulations, the next step in understanding how immune cells interact. Finally, the studies on hormone-immunocyte interactions are extremely important for understanding how to manipulate the immune response. In particular glucocorticoids are well known immunosuppressive agents whose clinical value is already empirically established. Once the nature of the susceptible cell types are delineated, the mechanism by which the hormones elicit their effects can be more specifically studied.

Proposed Course of Project: We are pursuing in greater depth the nature of the cells involved in the mixed lymphocyte interaction, direct cell-mediated cytotoxicity and hormone binding. In addition we are trying to extend the nylon column technique to the separation of human peripheral blood lymphocytes so that the same questions may be asked using human cells. Once the nature of the cells has been established, we hope to begin investigation of the biochemical events underlying cell-cell and cell-hormone interactions.

Honors and Awards: None

Publications:

Handwerger, B. S., and Schwartz, R. H.: Purification of murine bone marrow-derived and thymus-derived lymphocytes using nylon wool columns. *J. Immunol.*, submitted for publication.

Serial No. 5040(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Three-Dimensional Structure of Immunoglobulins

Previous Serial Number: None

Principal Investigator: David M. Segal, Ph.D.

Other Investigators: David R. Davies, Ph.D.; Eduardo A. Padlan, Ph.D.; Stuart Rudikoff, Ph.D.; Michael Potter, M.D.

Man Years:

Total:
Professional:
Other:

Project Description:

Objectives: To elucidate the stereochemical basis of antibody specificity and diversity, and to show how antigenic recognition leads to a given response in the immune system.

Methods Employed: Mouse myeloma proteins with binding activities toward natural antigens were purified by affinity chromatography, and their Fab fragments prepared by digestion with papain. Crystals of some of the Fab fragments were obtained from ammonium sulfate solutions, and two proteins gave crystals suitable for high resolution diffraction studies.

Crystalline protein-heavy atom complexes were prepared and electron density maps were calculated using standard X-ray diffraction techniques. A three-dimensional model of one protein has been constructed using Kendrew wire models and an optical comparator.

Major Findings: The structure of the Fab fragment of McPC603, a phosphorylcholine-binding IgA, has been determined to 3.1A° resolution. The hapten binding site has been located in a cleft formed by the three heavy chain hypervariable regions, and by the first and third hypervariable regions of the light chain. There are strong interactions between the phosphate group of the hapten and the side chain groups of tyr 33 and arg 52, both of the heavy chain. Acidic side groups from both light and heavy chains are in the immediate vicinity of the positively charged choline group. The hapten is in van der Waals contact with various portions of the hypervariable loops, but occupies only a small portion of the cleft between the variable domains.

Significance to Biomedical Research and the Program of the Institute: A key link in the major vertebrate (and therefore human) defense mechanism, i.e. the immune system, is the molecular recognition of foreign substances. The present study shows how a given protein molecule specifically interacts with a component found in the cell wall of a wide variety of pathogenic organisms. Moreover, we are currently working out the structure of another myeloma protein with specificity toward a different antigen. Taken together these two studies should give a good indication of how the immune system creates diversity on the molecular level, and what kind of interactions are involved in the recognition phenomenon. It is believed that our studies will give rise to a greater sophistication in both the understanding and control of the immune system.

Proposed Course of Project: We will continue to work out the structure of the second immunoglobulin, and will eventually compare the two immunoglobulin structures. We also plan to fit different amino acid sequence to the basic immunoglobulin structure to further our understanding of antibody diversity.

In addition I plan to begin chemical and X-ray studies on both the Fc fragment and whole immunoglobulins in order to gain information into the complement and B cell "triggering" mechanism.

Honors and Awards: None

Publications: None

LABORATORY OF CELL BIOLOGY

SUMMARY

July 1, 1973 - June 30, 1974

Lloyd W. Law

Some highlights of achievements in the Laboratory of Cell Biology

I. Tumor specific antigens in neoplasms of the experimental animal and tumor associated antigens of man and the immune reactions involved.

Studies of the biologic properties of several solubilized VSTA and TSTA continue. These are antigens solubilized from well-defined populations of neoplastic cells: RBL-5 leukemia containing RLV-specified antigen, mKSA containing an SV-40 specified antigen and Adj PC-5 containing a transplantation antigen presumably of spontaneous origin. The antigen located on the membrane of the plasma cell tumor Adj PC-5 was found to be extremely labile, in contrast to others we have studied and solubilization of this has not been continued.

Sensitive assays have now been used to characterize the soluble antigen on RBL-5 leukemic cells. The evidence is now clear that the antigen involved (VSTA) in tumor rejection is not infectious virus, budding from the cell surface nor is it viral envelope antigen; most likely it is a virus-specified new cellular antigen analogous to the VSTA of DNA virus-induced membrane antigens such as the SV-40 antigen on mKSA cells. To detect infectious type-C viruses possibly present in these preparations, NIH Swiss mouse embryo cells treated with DEAE-dextran were inoculated and after 8 days of cultivation were co-cultivated with XC cells, which acted as an indicator of infectious type-C viruses as shown by formation of syncytial cells. None of these preparations showed any infectious type-C viruses.

The syngeneic antiserum prepared in BL/6 mice by immunization with a mixture of crude membrane and complete Freund's adjuvant did not manifest any neutralization antibody activity against RLV or MSV(RLV) by XC plaque forming units or focus forming units reduction assay on NIH mouse embryo cell cultures. This would indicate that the crude membrane or crude soluble used for immunization contained little virion antigens capable of inducing detectable amount of neutralizing antibody.

A micro-complement fixation test was performed on the RBL-5 derived antigen preparations as well as on high titered RLV and MLV (Moloney leukemia virus) stocks which were prepared from mouse embryo cell culture supernates. These antigens were tested against broad-reacting antisera, such as Fischer rat anti-MSV(MLV) and hamster anti-RLV sera, which reacted with viral gs as well as envelope antigens. All antigen preparations except F1 showed the presence of virion antigens. The minimal concentrations in terms of protein μg/ml of antigens detectable by this method were 62-250 μg/ml. Two lots of syngeneic anti-crude membrane serum tested also reacted with these preparations with various degrees. It is interesting to note that the minimal concentration of F2 antigen detectable by these syngeneic antisera were very low, i.e. ≤ 1.8 µg/ml, whereas the minimal detectable antigen concentrations of other preparations were not so low. Such differential CF reactivity would suggest that the syngeneic antisera were detecting non-virion antigens beside virion antigens. Another lot of syngeneic antiserum showed a reactivity with crude soluble and F2 antigens without at the same time showing any reactivity with RLV or MLV. This would appear to indicate that the syngeneic antiserum contained an antibody which reacted exclusively with non-virion antigen which is present in crude soluble preparation and partially purified and concentrated in F2 preparation.

The immunological specificities of the activity of these RBL-5 antigens were indicated by the failure of the crude-membrane-immunized mice to be protected against the challenge by polyoma-specific neoplasm and by the failure of Gross virus induced syngeneic leukemic cells, E G2 to absorb from the syngeneic anti-RBL-5-crude membrane serum the CF antibody reacting with F2 antigen.

Further, the F2 preparation was the only fraction that contained a blocking activity against the complement-dependent cytotoxicity reaction of the syngeneic anti-RBL-5 crude membrane serum as assayed by Cr^{51}-release, or trypan blue staining techniques.

We conclude from these findings that a molecular entity with the characteristics of a tumor antigen of the transplantation type has been solubilized from a crude membrane preparation of RBL-5 cells, an RNA virus-transformed neoplasm. We now have a sensitive, simple, and reproducible _in vitro_ technique to monitor the tumor antigen for further purification and characterization.

Two points need to be stressed in this work:

1. Our published results provide the only instances of soluble, partially purified VSTA in which the immunogenic character is preserved and

2. The fact that both VSTA we have purified - from RBL-5 and from mKSA are recovered in the F2 fraction from G-150 Sephadex chromatography resemble characteristics of H-2 antigen. This raises the question that perhaps VSTA of the transplantation type are indeed modified histocompatibility antigens.

A search for TSTA of plasma cell tumors continues in a collaborative venture with Drs. McCoy and Dean of Bionetics Laboratory. The following findings are pertinent using a battery of different plasma cell neoplasms (PCT) in syngeneic BALB/c mice:

1. Not all PCT have detectable TSTA.

2. Some are relatively strong such as that described previously for Adj-PC5.

3. Some TSTA appear distinct (similar to MCA tumors); others appear common and show cross reactivity.

4. The TSTA of Adj-PC5 are extremely labile and are inactivated easily. Thus, if others of this series behave as the prototype Adj PC5, the likelihood of solubilizing and purifying is not good.

5. In several PCT systems it has been found that the immune response against TSTA, as revealed in the Winn assay, is effected by T-derived lymphocytes (sensitive to anti-θ serum).

During the past year we have continued to investigate the mechanisms involved in the immune rejection of syngeneic neoplasms, specifically the SV40 virus transformed tumor mKSA. A search for specific antibody to the tumor was made: no cytotoxic or lymphocyte dependent antibody could be found in immunized or tumor bearing mice, however, low levels of antibody were detected by the isotopic antiglobulin technique. The Winn assay (adoptive immunity) was used to study the cellular component of the rejection. With the use of anti-θ serum and mitogens, the Winn assay was shown to depend on T cells in the attacking population. When thymectomized, irradiated, bone marrow reconstituted mice were used as recipients they were capable of supporting a positive Winn assay, indicating that host T cells are not required in this rejection model. Lymphocytes from tumor bearing mice express CMI in the Winn assay during early tumor growth, but immunity wanes as the tumor becomes large. In contrast CMI expressed in the microcytotoxicity test (Hellström) was found to increase progressively with tumor growth. No serum factors capable of blocking the Winn assay were found in tumor bearing mice.

Investigations are continuing on tumor bearing mice in an effort to elucidate at what point in the rejection process lymphocytes from tumor bearing animals are blocked in their expression of cytotoxicity to the tumor. Although unreactive in the Winn assay, tumor bearing lymphocytes proliferate upon exposure to tumor specific transplantation antigens, and are capable of adoptively transferring systemic immunity to irradiated syngeneic recipients. Attempts will be made in the future to further dissect this suppression of CMI by tumor and recovery following tumor removal.

BALB/c mice bearing syngeneic SV-40 derived tumors for 0-4 weeks were tested for their cellular immune capacity using a combination of _in vivo_ and _in vitro_ assays. Early tumor growth was associated with detectable cellular immunity as measured by direct tumor challenge, the Winn test, and a modified microcytotoxicity assay. Animals bearing advanced tumors appeared to have lost immunity detected by direct tumor challenge and the Winn test, whereas specific immunity detected by the microcytotoxicity assay persisted. These studies support previous concepts that the immune status of tumor bearing mice is altered with increasing total tumor load. Additionally, the discrepancy between these assays performed in parallel may reflect functionally different components of the immune response to tumor-associated transplantation antigens.

Neoplastic cells induced by polyoma or SV-40 contain very strong TSTA of the rejection type. It was found that tumor Py 89 (polyoma) and mKSA (SV-40) when grown in vitro lose their tumor producing capacities but retain their capacities to immunize their syngeneic hosts. For example, 1 x 10^6 in vitro grown mKSA cells can immunize against 1 x 10^6 mKSA oncogenic ascites cells. A similar situation exists in relation to the Py 89 tumor except that here if challenge is made later than 3 weeks after immunization, a striking facilitation of tumor growth occurs. This system provides a model to study the kinetics of immunization versus facilitation (enhancement ?) in a syngeneic tumor system.

Cell-mediated immunity has been studied in the MSV system by the lymphocyte stimulation assay. It has been found that during the period of transient tumor growth, the ability of B cells to respond, even to mitogens, is markedly depressed. When the tumor regresses, reactivity returns to normal. With progressive growing tumors the defect remains marked until the death of the animals. This has been shown to be clearly due to the presence of a suppressor cell in the spleen, which appears to be an activated macrophage. Suppressor activity can be largely removed by treatment of the cells with carbonyl iron and removal of phagocytic cells by a magnet, or by passage of the cells over a rayon column. Before such treatment the spleen cells are poorly stimulated by mitomycin treated Rauscher or Moloney leukemia cells. After this treatment, better activity is seen.

Attempts have been made to better understand the various components of the cell-mediated immune response to MSV. George Klein and others had suggested that the principal effector cell reacting to H2 antigens was the T cell, but that to weaker tumor associated antigens, B cells constituted the major effector cell population. However, we clearly found that all of the cell-mediated cytotoxic reactivity, as measured by the ^{51}Cr release assay, could be eliminated by treatment with anti-theta and complement. The experiments with immunosuppression have been performed to further dissect out the nature of the cells involved in the cell-mediated immune response against MSV. Such studies were particularly appropriate because very little information is currently available on the effect of various chemotherapeutic agents on cell-mediated immune responses to tumor associated antigens. Almost all of the data obtained to date with these agents, both in experimental animals and in man, have been with other antigens. These experiments have provided evidence for differential effects of various agents on either the induction of immunity or more on the effector cells. There have been some interesting differences between the effects of some agents on tumor growth as opposed to their effects on in vitro reactivity measured in the ^{51}Cr release assay.

We have measured cell-mediated immunity to the W/Fu rat Gross virus-induced lymphoma (C58NT)D by the ^{51}Cr release cytotoxicity assay and by the mixed lymphocyte tumor interaction. We have compared kinetics of immune responses measured by these assays, and also the specificity of the antigens detected in each assay. The relationship of the reactivities measured in vitro to in vivo resistance against tumor growth, by performance of adoptive transfer experiments, was determined. Direct cytotoxic reactivity, as measured by the ^{51}Cr release assay, has shown that there is a peak of cytotoxicity at ten days after tumor cell inoculation with a rapid decline to negligible levels after 15 days. In contrast, the lymphocyte stimulation assay with mitomycin treated tumor cells has not become positive before 15 days, and reaches a maximum between 25 and 40 days. With progressive tumor growth the cytotoxic response is increased relative to the regressor system; in contrast the mixed lymphocyte tumor cell interaction in progressor rats is uniformly negative. Despite these marked differences in the kinetics in each assay, both responses have been shown to be due to T cells (complete inactivation by a specific anti-T cell serum plus complement). Studies of the specificity of the antigen detected in the cytotoxicity assay have given results similar to those obtained with the mouse MSV system. The antigen appears to be related to endogenous rat C-type virus.

A series of assays for measuring the general cell-mediated immune competence of cancer patients have been established. These assays are: enumeration of the percent of T cells in the peripheral blood by rosette formation with sheep erythrocytes, cytotoxic reactivity against human lymphoid cell lines, and lymphocyte stimulation by mitogens, antigens, and mixed lymphocyte cultures. A major problem with these assays for monitoring has been the day-to-day technical variations in the procedures. It has been possible to cryopreserve human lymphocytes and retain their functional activities in each of these assays. This has resulted in a considerable decrease in the variability of the assays and allows a consistent standard to be included in all of the assays.

Further studies have been performed with the direct leukocyte migration inhibition assay. The leukocytes of patients with carcinoma of the breast and with malignant melanoma have been shown to be specifically inhibited by hypertonic KCl extracts of autologous or allogeneic tumors of the same histological type. Reactivity has been found in patients with localized disease and also in patients with widespread, metastatic disease.

Lymphocyte stimulation assays have also been performed with these KCl extracts of tumors. It was found that it was not possible to use allogeneic extracts for measurement of tumor immunity, since these extracts also produced appreciable stimulation of many control individuals.

Patients with neoplastic disease have been shown to have delayed hypersensitivity reactions to extracts of tumor cells. Reactions to membrane extracts of tumor cells are being used to follow the course of disease of patients and correlations with clinical parameters are being made. The patients have been tested simultaneously by skin reactivity and by leukocyte migration inhibition assays. Thus far, these assays have shown good correlation; however, the skin tests have appeared to be more sensitive.

Soluble skin reactive antigens have been isolated from the membranes of breast tumors and of malignant melanoma. In each of these diseases, two separate skin reactive antigens have been isolated by polyacrylamide gel electrophoresis. One antigen has given reactions only in patients with the specific disease; the other antigen has shown wider reactivity, in patients with the particular disease and also in patients with other types of cancer. It seems likely that the latter antigen is a tissue associated antigen rather than tumor associated.

Experiments were designed to set up models for immunodiagnosis of cancer. CBF$_1$ mice were implanted in the foot pad with Trocar pieces of syngeneic SV40 tumor SVT$_2$. The tumors became grossly recognizable at two weeks after implantation; they were removed after three weeks in half of the mice, and tumors were left to grow in the other half. Sera were obtained from these mice at different intervals after tumor implantation and surgical excision. Testing by IAT (isotopic antiglobulin technique) it was found that anti-SV40 tumor antibody could be detected at two weeks after tumor implantation, which was the time that tumors became grossly recognizable. The level of antibodies rose with tumor progression and declined after amputation but remained detectable up to 11 weeks after implantation, when the experiment was terminated. Testing by ^{51}Cr cytotoxicity test (CT), antitumor antibody could be detected at four weeks after tumor implantation; the level of antibodies also rose with tumor progression but became undetectable at two weeks after amputation. In comparison of the sensitivity of the two serological techniques, IAT was found to be 100-400 times more sensitive than CT.

C57BL/6 mice were inoculated intraperitoneally with 1 x 10^2 FBL-3 cells, a syngeneic Friend leukemia, and DBA/2 mice were inoculated subcutaneously with 1 x 10[illegible] FLC cells, a syngeneic Friend leukemia. Antitumor antibodies to these tumor cells could be detected one-two weeks prior to tumor development or at the time of tumor development. The antibody levels rose with tumor progression.

Previous serological studies performed with IAT revealed that three antigenic specificities could be defined by the sera produced in C57BL/6 mice immunized with syngeneic Friend leukemia FBL-3. These specificities are: type specific (Friend) antigen, FMR common antigen, and a broadly cross reactive antigen of fetal nature. In vivo tumor transplantation experiments revealed that only the first two antigens were correlated with tumor associated transplantation antigen (TATA).

a. Correlation of the antigenic specificities defined by ^{125}IUDR assay, to those defined by serological studies and tumor transplantation experiments: Three antigenic specificities could be defined by ^{125}IUDR assay: (1) Lymphocytes from FBL-3 immune mice always gave higher degree (3-fold more) of cytotoxicity against FBL-3 than against leukemias induced by Rauscher virus (RBL-5) or Moloney virus (MBL-2) which suggested that there was a type specific (Friend) antigen in addition to FMR common antigen. (2) Occasionally, low grade cytotoxicity could be obtained against a chemical induced leukosis EL.4, which indicated a non-FMR cross reactive antigen. Therefore, these specificities correlated with the serological studies. The detection of cross reactive antigen by ^{125}IUDR assay indicated that either this assay was more sensitive than in vivo tumor transplantation experiments, or it was not correlated with TATA.

b. Specificity: The specificity of the reaction was revealed by the difference in reactivity of the FBL-3 immune lymphocytes against various leukemic cells as shown above. Furthermore, lymphocytes from EL.4 immune mice were generally not cytotoxic against FMR leukemic cells, but would give low grade of cytotoxicity against EL.4 cells. The difference in the reactivity of these cells could not be simple attributed to their susceptibility to lymphocyte cytotoxicity; lymphocytes obtained from mice immunized with Moloney sarcoma virus gave the same degree of cytotoxicity against FMR leukemias and EL.4 cells.

c. Kinetics of immune response: Significant cytotoxicity could be detected at five days after immunization, peaked at 10-14 days, then gradually declined but remained reactive up to 32 days or more.

d. Correlation of the lymphocyte cytotoxicity to status of tumor growth: Significant cytotoxicity to FBL-3 cells could only be obtained with lymphocytes from mice immunized with 5000 R x-irradiated FBL-3 cells, or immunized with viable FBL-3 cells by subcutaneous injection which only resulted in temporal tumor growth. No significant cytotoxicity was obtained with lymphocytes from tumor bearing mice. Therefore, the lymphocyte cytotoxicity measured by ^{125}IUDR assay seems to be correlated with the status of tumor growth.

Experiments were designed to study the expression of fetal antigens in fetal tissue culture cells, its relationship to spontaneous transformation, and the possible usefulness of these cells as the standard source of fetal antigens.

It was found that fetal antigens were retained in fetal tissue culture cells for up to five years of long term culturing. The expression of fetal antigens varied with cell lines and also varied with different strains of mive. There was found no correlation between the expression of fetal antigen and spontaneous neoplastic transformation.

Since fetal antigens are not found in normal adult tissue and some protection against a MCA-induced sarcoma in B6 mice could be achieved by immunization with fetal antigens, these tissue culture cells may be useful as the source of fetal antigen for immunodiagnosis or immunotherapy.

II. Histocompatibility Antigens

Work with solubilized, partially purified histocompatibility antigens continues . We have attempted to study the mechanism of immunologic tolerance to histocompatibility antigens (H-2) using the congenic strains of mice B10.A ($H-2^a$) and B10.D2 - the latter made 1) completely tolerant by transfer of B10.A lymphoid cells at birth and 2) humorally tolerant through inoculations from birth to weaning with $H-2^a$ soluble antigen. The classic conception of tolerance (Burnett, Medawar) is that clones of lymphoid cells are selectively deleted or inactivated under proper conditions when presented with antigen (central failure). More recently, particularly from the work of the Hellströms, it has been suggested that tolerance results from a peripheral interference of the immune response by the interposition of a serum factor (blocking). In collaboration with Dr. Peter Wright we have examined the role of serum obtained from our completely tolerant and humorally tolerant B10.D2 mice in blocking lymphoid cell cytolysis in a microcytotoxicity (48-72 hr assay) and in a ^{51}Cr (4 hour assay). It was found that serum from our operationally tolerant mice (with skin grafts) but not from our humorally tolerant mice did indeed block lysis of B10.A embryonic fibroblasts in the microcytotoxicity assay (used by the Hellströms) but did not block cell lysis in the short term ^{51}Cr release assay. Only hyperimmune antisera were capable of blocking in the latter system and only these sera had the capacity to selectively "enhance" A strain tumors in B10.D2 mice. Thus, the concept that serum blocking factors serve an in vivo role in allograft tolerance remains to be established if a more direct effect can be demonstrated.

We have investigated the cell lesion responsible for humoral tolerance in the H-2^{a} system by: 1) reconstituting thymectomized B10.D2 mice with T-cells (thymic origin) and with T-derived cells (lymph node origin) and 2) reconstituting lethally irradiated B10.D2 mice with B-cells (bone marrow origin and B-derived cells (splenic origin). Reconstituted mice stimulated with antigen (B10.A) were then assayed for their ability to produce cytotoxic antibody by a ^{51}Cr release assay. Preliminary evidence indicates that T-derived cells (not T cells) only are incapable of reconstituting the deficient recipients. This would suggest that those T-derived cells in lymph nodes are not capable of providing a "helper" function for B cells in antibody production.

Attempts to induce complete tolerance in the combination B10A → B10D2 using lymphoid cell inoculation at birth are difficult - only 10-20% of the recipients exhibit long lasting tolerance (cell-mediated). We have therefore attempted to induce complete tolerance in the direction B10.D2 → B10.A using soluble antigen from B10.D2 mice; cell-mediated tolerance here is easy to achieve (90%). Though some partial tolerance (complete) with soluble antigen is achieved the findings appear to indicate that cell-mediated tolerance cannot be achieved with soluble antigen even in an extremely sensitive system.

Congenic B10.Br recipients (differing from B10.A in many fewer determinants) are presently being used in attempts to understand why cell-mediated tolerance is not obtained with soluble antigen.

III. Studies in Immunochemistry

Studies on allotypic markers in rabbits (allotypes being antigenic determinants subjected to Mendelian segregation) have been extended to define the structural nature of these determinants. Peptides have been isolated from heavy and light chains which inhibited specific precipitation of anti-allotype antibodies. The structure of these peptides in terms of primary sequence and confirmation is being analyzed. An intensive program is being devoted to the purification of histocompatibility antigens and tumor specific antigens. The structural analysis of the purified HL-A systemin man has been started. One of the very interesting findings is the identity of the HL-A common portion fragment and human β_2-microglobulin. This combination seems to be specific and thus the common portion fragment or β_2 microglobulin must bear a definite function as a part of the HL-A molecule. We are now in a position to extend such studies to the H-2 system of the mouse and attempt to relate these findings to the biologic functions of our solubilized H-2 and tumor antigens.

Over the past year work has continued to determine the molecular structure of the antibody combining site. The crystallization and structure of the Fab of McPC 603 has been described as a phosphorylcholine binding myeloma protein at 4.5 Å resolution. The determination of the amino acid sequence of the entire variable region of the heavy chain from this protein has recently been completed. Approximately 80% of the light chain sequence has also been completed. X-ray data have been collected to 3.1 Å resolution and we are currently in the process of building a molecular model of this antibody combining site. Crystallization of the Fab from a galactan binding myeloma protein has been done and studies have been initiated as those described above which will eventually permit the comparison at the molecular level of the combining sites of two antibodies with quite different specificities.

In conjunction with the above study genetic variability among antibodies binding the same hapten have been studied. The NH_2 terminal sequences of 6 proteins which bind β (1→6) galactan and 4 proteins which bind phosphorylcholine have also been reported. Detailed structural studies on these groups of proteins are now being conducted.

Conclusive evidence of two species of phenylalanyl-tRNA in normal liver as well as in hepatoma tissue have been found. This casts considerable doubt on the literature reports of one of these Phe-tRNAs being "tumor-specific" or "tumor and fetal specific." We have preliminary evidence that the structural basis for the difference between these two tRNAs lies in the presence of a fluorescent base, "Y base," which seems to be missing in the minor Phe-tRNA. Investigation continues on whether the increased amount of this form found in tumors and fetal tissues compared to normal liver (in which it has previously thought to be completely absent) could be due to an arrest in the normal maturation of Phe-tRNA; i.e. tumors and embryos may not have enough of some enzyme to add "Y base" to all the Phe-tRNA, and this unsubstituted tRNA is seen as this minor peak.

The past year has been spent working on clinical application of sensitive quantitation of tumor markers found in the serum of individuals with various neoplastic diseases. The major emphasis has been with alpha-fetoprotein (αFP) but also has included human chorionic gonadotropin (hCG) and carcinoembryonic antigen (CEA). We have demonstrated the usefulness of αFP as a marker for gastric and pancreatic cancer by a large survey of patients with gastrointestinal cancer in collaboration with the Mayo Clinic. Work has also been done in the area of combining two markers to see what added benefit might derive. This has been shown to be of vital importance for mixed tumors of testicular origin, but also for gastric and pancreatic cancer. We have demonstrated the value of these markers for following patients to gauge the effectiveness of therapy and especially to predict the earliest time of tumor recurrence after remission.

IV. Studies in Somatic Cell Genetics

Evidence was obtained in collaboration with Igor B. Dawid and Ivan Horak, Carnegie Institution of Washington, Department of Embryology, Baltimore that a sexual system exists in mammalian mitochondria. At the same time a powerful methodology for studying the interaction of the mammalian nuclear and mitochondrial genome was established.

A new class of interspecific hybrid cells, those which segregate chromosomes in a heterodox fashion was discovered - including a reliable method of producing them. These reverse segregant hybrids complete the symmetry of the genetic system for vertebrate cells in culture and have implications for formal genetic analysis in rodents, for chromosome structure and inactivation, and provide unique material for studying human neoplastic and virus transformed cells in culture. In collaboration with Dr. John Minna (H:LBG) 20 isozymes have been assayed by starch gel electrophoresis in 25 reverse segregant human-mouse hybrids. In addition to confirming reverse segregation, evidence has been reported that HGPRTase, GPI, and PGK are syntenic in the mouse X chromosome as they are in man.

By application of the cellular hybridization technique developed in this laboratory an experimental strategem has been developed whereby mature neurons from the vertebrate nervous system can be selected and grown "clonally" in culture. If the immunological, neurochemical and cytogenetic analyses now underway confirm our initial observations, this system of neuron-fibroblast normally segregating hybrid cells will provide the material for extensive investigation in experimental neurobiology.

A new method has been developed utilizing reverse segregant exconjugants of mouse-human hybrid cells for producing human cell lines which are haploid (monosomic) for one-third to one-half of their chromosome complement. This material may be expected to make possible some of the first genetics analyzable by mutation of human cells in culture. These cell strains have potentially important application in the assay of chemical carcinogens and other mutagens. These strains may be the forerunner of fully haploid cell strains sought by extensive contract programs of the NCI.

A BUdR resistant avian cell line has been developed, HAT sensitive and deficient for the enzyme thymidine kinase. This cell line will make possible detailed study of inter-specific hybrids of birds (and their tumor viruses) as well as the first inter-class hybrids between avian and mammalian cells. These latter should be of great help in producing replicating haploid mammalian cell strains because of the high degree of incompatibility between mammalian and avian chromosomes in hybrid cells.

V. Studies in Viral Oncology, Ultrastructural Biology and Macromolecular Biology

The SJL/J strain of mouse which manifests a high incidence of spontaneous Hodgkin's like reticulum cell neoplasm, type-B (RCN-B) was studied to elucidate the possible role played by type-C RNA viruses in the pathogenesis of RCN-B in general.

It was found that the virus, designated D1-MuLV, which was isolated from a mouse bearing RCN-B, showed unique type-specific envelope antigens. By *in vitro* neutralization tests and immuno-electron microscopy, the viral envelope antigen (VEA) of this virus was shown to be distinct from the VEA of a number of different murine type-C viruses, including Gross, Friend, Moloney, Rauscher murine leukemia viruses (MuLV), or the MuLV associated with murine plasmacytomas and BALB/3T3 S2 (spontaneously transformed) cells. However, a cross-reacting VEA was found on a subpopulation of AKR-MuLV and a radiation-induced leukemia virus of A strain mice.

The antigen of D1 tumor, a lymphoblastic leukemia induced in SJL/J mice by neonatal inoculation of D1-MuLV, was also shown to be unique in that none of the virus-coded membrane antigens of the cell, such as GCSA or FMR, or alloantigens PC1 and G_{ix} associated with MuLV infection was present on the D1 cell surface.

These findings contributed to the development of an important concept that there existed in various mouse strains a wide variety of type-C viruses antigenically different from each other. This concept is in contrast to the commonly held view that all type-C viruses spontaneously arising in murine tissues and tumors are the same or variants of Gross-MuLV.

Pathogenicity studies of D1-MuLV also indicated some distinctive properties of this virus. When inoculated into neonatal SJL/J and AL/N mice, lymphoblastic leukemias were induced. Further studies indicated that RCN-B was induced in high frequency (30%) in BALB/c mice that had been neonatally thymectomized and inoculated with D1-MuLV. An unexpected finding in this experiment was that a large amount of an MuLV with B-tropism and envelope antigens different from those of D1-MuLV was recovered from the neoplastic tissues of those mice that developed RCN-B in 5 months after inoculation. Thus a possible activation of the endogenous type-C virus is strongly suggested. It is important to note that young BALB/c mice under 6 months of age show very low frequency of infectious type-C virus in their tissues, and that the virus recovered was B-tropic rather than N-tropic, since most of the virus isolates from BALB/c mice under 12 months of age were N-tropic. Such an activation of covert viral genome in vivo by infection with another virus of different tropism opens a new possibility of elucidating viral oncogenesis.

Further characterization of murine intracisternal A particles have been made. Analysis of the intrinsic RNA-dependent DNA polymerase indicates that it has many similarities to other viral reverse transcriptases. Demonstration of a complete endogenous reaction requires dithiothreitol, Mg, Na or K, and all four deoxyribonucleoside triphosphates. RNase digestion experiments indicated that this DNA polymerase was most probably dependent on the intrinsic A particle RNA as template. The products of the enzyme reaction were synthesized as follows. At the end of 5 minutes the ^{3}H-DNA was clearly linked to the endogenous RNA, but at the end of 30 minutes the product was found to be primarily free DNA. When this DNA was hybridized to the purified RNAs from intracisternal A particles, 69% hybridized to the 70S RNA and 88% to the 35S RNA. Now that a DNA probe is available, we are examining its homology with that of the high molecular weight RNAs of several of the C-type oncornaviruses. Information derived from such experiments will help demonstrate more definitively the genetic relationship of intracisternal A particles to other RNA tumor viruses.

We are studying A particles which are replicated during early embryogenesis. It has now been established that these particles are seen in small numbers in dictyate oocytes immediately after their release from ovarian follicles. They were not found in oocytes which reached metaphase II or in fertilized eggs. At the 4 cell stage large numbers of complete particles reappear within the endoplasmic reticulum. By the 16 cell stage and the 32+ cell stage (blastocyst) A particles are only rarely encountered. Current efforts are directed toward comparing the embryogenic A particle antigens with those of A particles from murine tumors, using immuno-electron microscopy.

Viral DNA polymerases are being studied in a number of different systems. One such system is a simian tissue culture cell line derived from a lymphoma induced in the owl monkey by Herpesvirus saimiri. Initially the cells yielded H saimiri in vitro, but virus production ceased after the first few passages. Later there was the spontaneous appearance of a membrane-bound RNA-dependent DNA polymerase which possesses many biochemical properties analogous to those of primate and murine RNA tumor viruses. Characteristics of this reverse transcriptase are clearly distinct from those of the DNA polymerase of H. saimiri Since this enzyme activity has features of a viral gene product, its relationship to the primate RNA tumor viruses or to H. saimiri merits additional investigation.

Work continues with the enzyme L-asparaginase from E. coli B, which is enzymatically and chemotherapeutically active when the subunits are associated in the tetramer form. By application of differential spectroscopy and ultracentrifugation the substrate binding site has been found which is rich in glutamyl and tyrosyl residues. Since the active tetramer can be maintained in the presence of the substrate even under conditions that are normally detrimental to the oligomers, the above residues are not modified when new asparaginase derivatives are being prepared and tested in an effort to lower toxicity but maintaining or increasing therapeutic effectiveness. It has also been found that glycerol and other polyhydroxy compounds are involved in a protective action for the enzyme activity, by preventing dissociation of the L-asparaginase subunits under otherwise disruptive conditions.

A systematic exploration has been initiated of the biosynthetic pathways leading to the terminal sialic acid residue of glycoproteins in the outermost surface of cells. With fluorinated and iodinated N-acetyl-glucosamine it has been found that these halogen derivatives are effective metabolic modifers of the amino-sugars which are converted to the sialic acid. By judicious selection of the mode of application and the dose of these halogen derivatives non-toxic doses and certain conditions exist when these derivatives can substantially completely depress (block), or in other conditions and doses when the derivatives can greatly increase, the synthesis and thus the appearance of cell surface sialic acid residues. The roles these substances play in cell interactions, neoplasia and tumor rejection may now be analyzed.

In collaborative experiments with Drs. W. P. Rowe and M. D. Sklar of the NIAID a rapid method for the induction of plasmacytomes in BALB/c mice has been developed. Twenty to 30% of BALB/c mice given a single injection of 0.5 ml pristane followed by Abelson virus in 30 to 40 days develop plasmacytomas, that produce immunoglobulins predominantly of the IgA class. In addition the mice also develop the typical Abelson lymphosarcomas. The earliestplasmacytomas appear 65 to 80 days after the injection pristane and well before i.e. the first tumors appear after the injection of pristane alone which begins 138 days after the injection of pristane. Thus far susceptibility follows the same genetic pattern observed with pristane alone i.e. BALB/c remains the only susceptible prototype strain. The method is currently being pursued to identify the basis for susceptibility.

E. B. Mushinski in this laboratory has completed the development of two new congenic strains of BALB/c: CAL-20 and CB-20; the AL/N and C57BL/Ka IgC_H loci were introduced onto BALB/c during 20 consecutive backcrosses. At the 20th backcross the mice were made homozygous for the introduced IgC_H loci. The introduced IgC_H locus contains at least 4 heavy chain genes.

The complex IgA heavy chains locus apparently also contains IgV region genes because we have now demonstrated that the congenic mice CB-2 and CAL-20 differ from BALB/c in immune responses. In collaborative experiments with Rose Lieberman we have been able to demonstrate that the gene that controls the heavy chain V-region of the TEPC 15 myeloma protein is linked to the IgC_H complex locus. The TEPC 15, an IgA phosphorylcholine binding myeloma protein, has the same idiotype (IgV region antigenic determinant) as natural occuring antiphosphorylcholine IgM antibodies in normal BALB/c serum. This idiotype marker is lacking in C57BL mice and CB-20 mice. The idiotype marker also behaves as a classical Mendelian gene in appropriate crosses between BALB/c and C57BL. The congenic mice have been useful in demonstrating other IgV-genes governing anti-dextran NIP, azophenylarsonate, antibodies.

Serial No. NCI-3200
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Factors influencing the induction and repression of primary tumors and the growth and inhibition of transplantable neoplasms. (Host and tumor cell variables)

Previous Serial Number: Same

Principal Investigator: Dr. Lloyd W. Law

Other Investigators:
Dr. E. Appella (NCI-3229)
Dr. Kenneth Chang (NCI-5550)
Dr. James McCoy (Bionetics)
Dr. Jack Dean (Bionetics)
Dr. Samuel Strober (Stanford University School of Medicine, Stanford, Calif.)
Dr. Stephen Howell (NCI-5560)
Dr. Barry Fernbach
Dr. Peter Wright (Univ. of Washington School of Medicine, Seattle, Washington)

Man Years:
Total: 10 1/2
Professional: 4
Other: 6 1/2

Project Description:

Objectives: The objectives of this project are: (1) Studies of the biologic properties of solubilized, partially purified (by G-150 Sephadex fractionation) $H\text{-}2^a$ antigens and virus-specified tumor antigens of the transplantation type (VSTA).

a) The biochemical methods used for solubilization and purification have shown preservation of most of the biologic functions of $H\text{-}2^a$ such as the capacity to inhibit antibody cytotoxicity to immunize and to achieve immunologic enhancement; immunologic tolerance as induced in neonates or B10.D2 congenic mice is restricted to humoral tolerance. Neonates receiving 40 or 0.4 μgms (but not 4 μgm) of the purified F2 fraction from birth through 4 weeks are unable to produce cytotoxic, hemagglutinating and enhancing antibodies but nevertheless retain the capacity to reject B10.A skin and heart grafts. Other T-cell functions were preserved or even facilitated. Those studied were GVH, reactivity in mixed leukocyte cultures and CML (cell mediated lymphocytotoxicity).

Work with solubilized, partially purified histocompatibility antigens continues. We have attempted to study the mechanism of immunologic tolerance to histocompatibility antigens (H-2) using the congenic strains of mice B10.A ($H-2^a$) and B10.D2 - the latter made 1) completely tolerant by transfer of B10.A lymphoid cells at birth and 2) humorally tolerant through inoculations from birth to weaning with $H-2^a$ soluble antigen. The classic conception of tolerance (Burnett, Medawar) is that clones of lymphoid cells are selectively deleted or inactivated under proper conditions when presented with antigen (central failure). More recently, particularly from the work of the Hellströms, it has been suggested that tolerance results from a peripheral interference of the immune response by the interposition of a serum factor (blocking). In collaboration with Dr. Peter Wright we have examined the role of serum obtained from our completely tolerant and humorally tolerant B10.D2 mice in blocking lymphoid cell cytolysis in a microcytotoxicity (48-72 hr assay) and in a ^{51}Cr (4 hour assay). It was found that serum from our operationally tolerant mice (with skin grafts) but not from our humorally tolerant mice did indeed block lysis of B10.A embryonic fibroblasts in the microcytotoxicity assay (used by the Hellströms) but did not block cell lysis in the short term ^{51}Cr release assay. Only hyperimmune antisera were capable of blocking in the latter system and only these sera had the capacity to selectively "enhance" A strain tumors in B10.D2 mice. Thus, the concept that serum blocking factors serve an in vivo role in allograft tolerance remains to be established and a more direct effect can be demonstrated.

We have investigated the cell lesion responsible for humoral tolerance in the $H-2^a$ system by: 1) reconstituting thymectomized B10.D2 mice with T-cells (thymic origin) and with T-derived cells (lymph node origin) and 2) reconstituting lethally irradiated B10.D2 mice with B-cells (bone marrow origin and B-derived cells (splenic origin). Reconstituted mice stimulated with antigen (B10.A) were then assayed for their ability to produce cytotoxic antibody by a ^{51}Cr release assay. Preliminary evidence indicates that T-derived cells (not T cells) only are incapable of reconstituting the deficient recipients. This would suggest that those T-derived cells in lymph nodes are not capable of providing a "helper" function for B cells in antibody production.

Attempts to induce complete tolerance in the combination B10A → B10.D2 using lymphoid cell inoculation at birth are difficult - only 10-20% of the recipients exhibit long lasting tolerance (cell-mediated). We have therefore attempted to induce complete tolerance in the direction B10.D2 →B10A using soluble antigen from B10.D2 mice; cell-mediated tolerance here is easy to achieve (90%). Though some partial tolerance (complete) with soluble antigen is achieved, the findings appear to indicate that cell-mediated tolerance cannot be achieved with soluble antigen even in an extremely sensitive system.

Congenic B10.Br recipients (differing from B10.A in many fewer determinants) are presently being used in attempts to understand why cell-mediated tolerance is not obtained with soluble antigen.

b) Studies of the biologic properties of several solubilized VSTA and TSTA continue. These are antigens solubilized from well-defined populations of neoplastic cells: RBL-5 leukemia containing RLV-specified antigen, mKSA containing an SV-40 specified antigen and Adj PC-5 containing a transplantation antigen presumably of spontaneous origin. The antigen located on the membrane of the plasma cell tumor Adj PC-5 was found to be extremely labile, in contrast to others we have studied and solubilization of this has not been continued.

Sensitive assays have now been used to characterize the soluble antigen on RBL-5 leukemic cells. The evidence is now clear that the antigen involved (VSTA) in tumor rejection is not infectious virus, budding from the cell surface nor is it viral envelope antigen; most likely it is a virus-specified new cellular antigen analogous to the VSTA of DNA-virus-induced membrane antigens such as the SV-40 antigen on mKSA cells (See report of K.S.S. Chang.).

Two points need to be stressed in this work: 1) our published results provide the only instances of soluble, partially purified VSTA in which the immunogenic character is preserved and 2) the fact that both VSTA we have purified - from RBL-5 and from mKSA are recovered in the F-2 fraction from G-150 Sephadex chromatography resemble characteristics of H-2 antigen. This raises the question that perhaps VSTA of the transplantation type are indeed modified histocompatibility antigens.

2. Studies of the immune response to different tumor antigens.

a) A search for TSTA of plasma cell tumors continues in a collaborative venture with Drs. McCoy and Dean of Bionetics. The following findings are pertinent using a battery of different plasma cell neoplasms (PCT) in syngeneic BALB/c mice:

1). Not all PCT have detectable TSTA.
2). Some are relatively strong such as that described previously for Adj-PC5.
3). Some TSTA appear distinct (similar to MCA tumors); others appear common and show cross reactivity.
4). The TSTA of Adj-PC5 are extremely labile and are inactivated easily. Thus if others of this series behave as the prototype Adj-PC5, the likelihood of solubilizing and purifying is not good.
5). In several PCT systems it has been found that the immune response against TSTA, as revealed in the Winn assay, is effected by T-derived lymphocytes (sensitive to anti-θ serum).

b) Neoplastic cells induced by polyoma or SV40 contain very strong TSTA of the rejection type. It was found that tumor Py 89 (polyoma) and mKSA(SV-40) when grown in vitro lose their tumor producing capacities but retain their capacities to immunize their syngeneic hosts. For example 1 x 10 in vitro grown mKSA cells can immunize against 1 x 10^6 mKSA oncogenic ascites cells. A similar situation exists in relation to the Py 89 tumor except that here if challenge is made later than 3 weeks after immunization, a striking facilitation of tumor growth occurs. This system provides a model to study the kinetics of immunization versus facilitation (enhancement ?) in a syngeneic tumor system.

c) BALB/c mice bearing syngeneic SV-40 derived tumors for 0-4 weeks were tested for their cellular immune capacity using a combination of in vivo and in vitro assays. Early tumor growth was associated with detectable cellular immunity as measured by direct tumor challenge, the Winn test, and a modified microcytotoxicity assay. Animals bearing advanced tumors appeared to have lost immunity detected by direct tumor challenge and the Winn test, whereas specific immunity detected by the microcytotoxicity assay persisted. These studies support previous concepts that the immune status of tumor bearing mice is altered with increasing total tumor load. The discrepancy between these assays performed in parallel may reflect functionally different components of the immune response to tumor-associated transplantation antigens.

d) During the past year we have continued to investigate the mechanisms involved in the immune rejection of syngeneic neoplasms, specifically the SV-40 virus transformed tumor mKSA. A search for specific antibody to the tumor was made: no cytotoxic or lymphocyte dependent antibody could be found in immunized or tumor bearing mice, however, low levels of antibody were detected by the isotopic antiglobulin technique. The Winn assay (adoptive immunity) was used to study the cellular compound of the rejection. With the use of anti-θ serum and mitogens, the Winn assay was shown to depend on T cells in the attacking populations. When thymectomized, irradiated, bone marrow reconstituted at what point in the rejection process lymphocytes from tumor bearing animals are blocked in their expression of cytotoxicity to the tumor. Although unreactive in the Winn assay, tumor bearing lymphocytes proliferate upon exposure to tumor specific transplantation antigens, and are capable of adoptively transferring systemic immunity to irradiated syngeneic recipients. Attempts are being made to further dissect this suppression of CMI by tumor and recovery following tumor removal.

Significance for Cancer Research:

Characterization of tumor antigens of the rejection type is a necessary prerequisite for understanding the mechanisms , immune surveillance, tumor inhibition and facilitation. In addition the role of these membrane bound components in the process of carcinogenesis may be studied.

As a basis for any study of membrane bound antigens it is necessary to study the nature of histocompatibility antigens and their relationship to tumor antigens. Thus our emphasis is on studies of H-2 antigens.

Objective 3; Approaches 4,5.

Publications:

Law, L. W.: Immune suppression and the induction of leukemia and solid tumors. Bibliothea Haematologica 39: 662-670, 1973.

Law, L. W. and Takemoto, K. K.: Specific transplantation antigens of murine neoplasms induced by visna and progressive pneumonia viruses. J. Nat. Cancer Inst. 50: 1075-1079, 1973.

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Drapkin, M., Appella, E. and Law, L. W.: Immunogenic properties of a soluble SV-40 induced tumor specific transplantation antigen (TSTA). J. Nat. Cancer Inst. 52: 259-264, 1974.

Chang, Kenneth S. S., Law, L. W. and Aoki, T.: Type-C RNA virus isolated from an SJL/J strain mouse. J. Nat. Cancer Inst. 52: 777-784, 1974.

Cohen, J. M. and Law, L. W.: Spontaneous disappearance of tumorigenicity in a polyoma virus induced neoplasm carried in vitro. J. Exp. Biol. Med. 145: 385-388, 1974.

Cohen, J. M., Yang, S. S., and Law, L. W.: Abrogation of cell-mediated immunity by hyperimmune alloantiserum: Mechanisms and correlation with allograft enhancement. Int. J. Cancer. In press.

Law, L. W., Chang, K. S. S. and Nakata, K.: Heterogeneity of virus production and antigenicity detected in cell clones derived from a "non-producer" neoplasm induced by Moloney murine sarcoma virus. J. Nat. Cancer Inst. 52: 437-443, 1974.

Law, L. W., Appella, E., Cohen, J. and Dean, J. H.: Reactivity of lymphoid cells from mice humorally tolerant to histocompatibility antigens. Nature 246: 174-176, 1973.

McCoy, J. L., Dean, J. H., Law, L. W., Williams, J., McCoy, N. T. and Holiman, B. J.: Immunogenicity, antigenicity and mechanisms of tumor rejection of mineral oil-induced plasmacytomas in syngeneic mice. Int. J. Cancer. In press.

McCoy, J. L., Ting, R. C., McCoy, N. T., Reischer, J. I., Shan, S. and Law, L. W.: Cell mediated and humoral immunity studies of murine sarcoma virus induced producer and non-producer tumors in syngeneic animals. Int. J. Cancer. In press.

Wright, P. W., Law, L. W., Appella, E. and Bernstein, I. O.: Absence of serum blocking activity in mice immunologically tolerant to soluble histocompatibilty antigens. Transplantation. In press.

Law, L. W., Appella, E., Cohen, J. M. and Wright, P. W.: Role of serum factors from tolerant mice in blocking and in immunologic enhancement. Transplantation. In press.

Howell, S. B., Esber, E. C., Law, L. W.: Cellular immunity in mice bearing SV-40 induced mKSA tumors: Comparison of three assays of tumor immunity. J. Nat. Cancer Inst. In press.

Serial No. NCI-3222
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Genetic and biochemical mechanisms controlling protein synthesis in mouse plasma cell tumors and other well-differentiated neoplastic cells

Previous Serial Number: Same

Principal Investigator: J. Frederic Mushinski, M.D.

Other Investigators:
Dr. Michael Potter (NCI-5556)
Dr. Carl Hansen (DRS-URB-5)
Dr. P. O. Perlman, George Washington University
Dr. Matthew D. Scharff, Albert Einstein School of Medicine
Dr. I. Bernard Weinstein, Columbia University
Dr. Dezider Grunberger, Columbia University

Cooperating Units:
George Washington University
Albert Einstein School of Medicine
Columbia University

Man Years:
Total - 2.00
Professional - 1.00
Other - 1.00

Project Description:

A) Objectives

To study mammalian control mechanisms in transcription, translation, and protein assembly using rodent tumors *in vivo* and *in vitro* as models of human neoplastic and normal cells.

B) To develop new animal models of immunoglobulin synthesis and secretion in inbred rats.

Methods Employed:

A. Control Mechanisms

1. IgA half molecule mouse myeloma proteins

Three approaches are being used to understand the genetic and biochemical events responsible for the synthesis and secretion of IgA half molecules, which consist of one light chain disulfide bonded to one unusually short, heavy chain, as seen in seven mouse plasmacytomas:

a. Agar cloning of IgA plasmacytoma cells to detect mutants which secrete half molecules detected by overlaid antiserum.

b. Protein structural studies on the seven examples of this unusual immunoglobulin to determine similarities within this group and differences from polymerizing IgA's.

c. Transfer RNA comparisons by reversed phase chromatography and cell-free protein synthesis.

2. Transfer RNA from mouse myelomas and rat hepatomas

The striking variation in isoaccepting tRNAs among tumors is being studied using the high resolving power of reversed phase, high speed liquid chromatography combined with BD-cellulose chromatography.

B. Induction of Rat Tumors

1. Intraperitoneal injections of mineral oil or Pristane induce a high incidence of plasmacytomas in BALB/c and NZB mice. We have injected 19 different inbred strains of rats with Pristane sometimes supplemented with burro antithymocyte serum in hopes of finding a rat strain which could be induced to develop plasmacytomas.

2. Rat and mouse viruses suspected of inducing leukemias, lymphomas, or sarcomas were also inoculated into newborn rats in attempts to induce plasmacytomas.

3. Another attempt to induce rat tumors utilized subcutaneous and intraperitoneal injections of aqueous lead acetate solution in inbred Sprague-Dawley rats.

Major Findings:

A. Control Mechanisms:

1. With a single exception, the seven IgA half molecules appear to have similar molecular weights by SDS-polyacrylamide gel electrophoresis, which means that they have heavy chains of 45,000 daltons. Carboxyl-terminal analysis of these heavy chains has been begun and the two analyzed so far both have glutamine as carboxyl-terminus [polymerizing IgA's have tyrosine as the carboxyl-terminus of the heavy chain]. Automatic sequenator studies of the amino-terminus of these heavy chains is also underway, but the initial trials have shown blocked amino-termini for a half molecule IgA heavy chain as well as for its parental polymerizing IgA heavy chain.

2. Utilizing RPC-5 reversed phase chromatography, repeated studies have firmly established the presence of an early-eluting peak of Phenyl-

alanyl-tRNA(Phe-tRNA$_1$) in normal, regenerating, and fetal rat liver. Phe-tRNA$_1$ has heretofore been thought to be present only in hepatomas or embryonic liver. Phe-tRNA$_1$ can only be detected in normal liver tRNA when a crude aminoacylating enzyme (G-10 enzyme) is used to charge phenylalanine to it and is not revealed when a more purified preparation (DEAE enzyme) is used for amino-acylation. It is still unclear what is lacking in the DEAE enzyme that is present in G-10 enzyme enabling it to aminocylate the early peak. Probably there are two Phe-tRNA synthetase enzymes which are separated by the DEAE cellulose chromatography. One is adsorbed to the DEAE cellulose and can charge only Phe-tRNA$_2$; the unbound enzyme is less restrictive in substrate activity and can aminoacylate both Phe-tRNA$_1$ and Phe-tRNA$_2$.

Considerable progress has been made in determining the structural basis for the difference between Phe-tRNA$_1$ and Phe-tRNA$_2$ from rat liver. Phe-tRNA$_1$ can be generated from Phe-tRNA$_2$ by mild acid hydrolysis at pH2.9, a treatment known to specifically hydrolyze an unusual, minor, fluorescent base (Base Y) from tRNA^{Phe}. From this finding as well as the less tight binding of Phe-tRNA$_1$ to RPC-5 and BD-Cellulose columns, which bind hydrophobic molecules best, we suggest that Phe-tRNA is lacking in Base Y. Either it is completely absent due to inadvertent exposure to acid during preparation (highly unlikely) or it may represent a form of tRNA^{Phe} upon which the full Base Y side chain has not yet been completely built up.

tRNA isolation procedures differ from laboratory to laboratory, and only rat liver tRNA from this laboratory shows Phe-tRNA$_1$. Rat liver tRNA from Columbia University contains only Phe-tRNA$_2$, even when aminoacylated at NIH with G-10 enzymes from this laboratory. The only salient difference between the two preparations is that cell nuclei may not be extracted in Columbia but are extracted here. Although we lack direct evidence proving this hypothesis, we feel that a nuclear tRNA, possibly an immature tRNA^{Phe} or a precursor, may be the source of Phe-tRNA$_1$. tRNA from any of three Morris hepatomas show significant enrichment for Phe-tRNA$_1$ compared to normal liver. We feel that the tumor tRNA may have an abnormally large amount of this type of tRNA, compared to normal liver, reflecting a maturation arrest in these neoplastic cells.

B. Induction of Rat Tumors

1. Eighteen months have elapsed since the initial Pristane and antithymocyte serum injections. We have detected no plasmacytoma cells by examination of stained smears of ascites nor myeloma proteins by cellulose acetate electrophoresis of ascites or serum from the injected animals.

An intriguing finding is that many of the uninjected, control rats have spontaneously developed tumors, most noticeably mammary fibroadenomas. Strikingly fewer of the animals receiving Pristane or Pristane plus antithymocyte serum have developed such tumors. This observation is complicated by the much greater death rate in those rats which had received Pristane.

2. No plasmacytomas were induced by any of the rodent viruses administered to newborn inbred rats. Abelson virus, from Dr. Richard Siegler of the Childrens Cancer Research Foundation, Inc. Boston, Mass. regularly induced anemia, fluid filled cysts in lymph nodes, but no solid tumors.

3. Subcutaneous fibrosarcomas developed at the site of lead acetate injections in 7 out of 7 male and 3 out of 5 female inbred Sprague-Dawley rats. The males developed tumors significantly earlier then the females. 8 out of 8 tumors tested were transplantable to other inbred Sprague-Dawley animals, male or female, but not to non-inbred Sprague-Dawley rats nor to adult rats of seven other inbred strains. When injected as newborns, allogeneic rats developed tumors when as few as 750 cells from one of the Sprague-Dawley tumors were inoculated subcutaneously. These tumors, however, were rejected at age 9-10 weeks when the hosts became immuno competent. Injections of magnesium acetate caused no tumor development. No kidney tumors were seen as reported for Wistar and Chester-Beatty rats injected with subcutaneous lead salts.

Significance to Biomedical Research and the Program of the National Cancer Institute:

A. The recent report of an IgA half-molecule from a human myeloma patient broadens the interest in our studies of this unusual type of mouse myeloma protein. Continued structural and related studies will indicate whether this type protein is a normal but rare subtype of IgA in mouse and/or man. Recent findings by Dr. Scharff that mutant clones of IgG-producing plasmacytomas produce heavy chains of abnormal length add promise to this approach to understanding the IgA half molecule problem, which has so far not been fruitful due to difficulty in cloning IgA producing plasmacytomas in soft agar.

The Phe-tRNA findings reported here appear promising in explaining not only the differences between normal and neoplastic Phe-tRNA profiles but also the differences between the published data from other laboratories and that of our laboratory. It is important to establish if a Base Y deficiency exists in nuclear or immature $tRNA^{Phe}$, for we suspect from preliminary experiments that this may lead to codon recognition differences. Such coding abnormalities could produce neoplastic changes in metabolism or growth.

B. The failure of intraperitoneal Pristane to induce plasma cell tumors in any of 18 inbred rat strains reemphasizes how special the BALB/c and NZB mouse strains are in their genetic susceptibility to this type of carcinogensis. Despite our extensive efforts at this induction, the only examples of plasmacytomas in rat are those which arise spontaneously in the LOU strain of rats.

The injection site sarcomas appear to be a strain-specific reaction to subcutaneous aqueous lead salts, since published studies of lead acetate injections in other strains report only kidney tumors. Since lead and other heavy metals are well-known environmental pollutants, these results show that individuals may exist who have a genetic susceptibility to develop cancer at the site of deposition of lead salts. Since malignancies may develop at sites of exposure, lead compounds can be harmful even if not absorbed by the body.

Proposed Course

A. Continuation and intensification of structural studies of the IgA half molecules and related IgA polymerizing molecules will point out how similar the mechanism of generation of these shortened heavy chain is for all the examples we have to study. A sensitive radioimmunoassay will be developed using our specific anti-half molecule antiserums to search for this IgA type in normal mouse serum and body fluids.

Continuation and intensification of biological and structural studies of the rat Phe-tRNAs and their syntheteses will clarify further the site of origin of Phe-tRNA$_1$ in normal and neoplastic liver cells. Polynucleotide ribosome binding of the different peaks of Phe-tRNA will show how Base Y, intact or incomplete, affects the codon recognition of tRNA^{Phe}. The use of a Base Y-specific antibody developed in the laboratory of Drs. Fuchs and Sela in Israel may prove useful in confirming our conclusions about the presence or absence of Base Y in the several peaks of Phe-tRNA we have separated.

B. We propose an orderly termination of the Pristane trials at plasmacytoma induction in rats. Comparative hematologic and pathologic results of this treatment will be determined when the surviving animals are sacrificed.

The lead acetate induced series of fibrosarcomas are frozen down for future study. We are now engaged in sequential biopsies of lead acetate injected rats in hopes of delineating the pathogenesis of these tumors.

Relationship to National Cancer Plan:

Objective - 3

Approach - 1

Publications:

Mushinski, J. F.: Hepatoma-foetal Phe-tRNA also present in normal rat liver. Nature 248: 232-234, 1974.

Mushinski, J. F.: Mouse IgA myeloma proteins with alpha chain deletions: A comparison with human alpha chains with deletions. In Mestecty, J. (ed.): International Symposium on the Immunoglobulin A System. New York, N. Y., Plenum Press. (in press).

Serial No. NCI-3229
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: I. Chemical structure of immunoglobulin molecules and allotypes
II. Purification and biochemical characterization of soluble murine H-2 antigens, human HL-A antigens, and tumor specific antigens

Previous Serial Number: Same

Principal Investigator: Dr. Ettore Appella

Other Investigators: Dr. R. Mage (NIAID)
Dr. John Inman (NIAID)
Dr. N. Tanigaki, Roswell Park Memorial Inst., Buffalo
Dr. O. Roholt, Roswell Park Memorial Inst., Buffalo
Dr. D. Pressman, Roswell Park Memorial Inst., Buffalo

Man Years:

Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

RABBIT IMMUNOGLOBULINS

Studies on the structural basis of antibody specificity have been carried out labeling the antibody combining site of an anti-p-azobenzoate antibody of restricted heterogeneity. Using the paired-iodination technique, tyrosine residue at position 96 of the light chain was found to contain most of the label. These studies have been extended to a second anti-p-azobenzoate antibody. Labeled tyrosine peptides were isolated in good yield from the light chain. The definitive assignment of the position of the labeled tyrosine has not yet been obtained but complete sequence analysis of this light chain is being carried out. The comparison of the sequence of these two light chains will provide some insight into the type of variability of the site and the possible mechanism involved. Contrary to immunoglobulins of all other species, rabbit IgG, IgA, IgM and IgE carry allotypic specificities which are alleles at a single locus. These alleles, $\underline{a}_1$, $\underline{a}_2$ and $\underline{a}_3$, correlate with sequence variations within the amino-terminal 33 positions of the heavy chain and possibly within section 80 to 85. The presence of these segregating structural markers in the above position is difficult to reconcile with a multiplicity of genes responsible for variable region synthesis unless specific mechanisms are postulated. The definition of the structural correlates of these allotypic specificities is crucial for the selection among various hypotheses. We have taken the approach of isolating peptides from rabbit heavy chains of each a-locus allotype which inhibited specific precipitation of anti-a-locus antibodies. Active peptides were highly purified by the use of immunoabsorbent columns. By an agarose column calibrated for the determination of molecular weights it has been found that the active peptides consist of two fragments of about 30 amino acid residues linked by a disulfide bridge. We are not yet certain of the exact localization of these fragments in the sequence of the heavy chain, but these studies are being pursued. The isolation and characterization of active peptides from the rabbit light chain is also a study that is actively being pursued.

HISTOCOMPATIBILITY ANTIGENS

Histocompatibility antigens are cellular products of histocompatibility genes and carry the immunological determinants involved in tissue transplant rejection. In the mouse the main histocompatibility system is the H-2, in the human the HL-A. The genes controlling these antigens exhibit an extraordinary degree of polymorphism. More than 25 different haplotypes are known in the mouse and approximately 34 specificities have been recognized in humans. A knowledge of the structure of the H-2 and HL-A systems is of key importance for understanding their genetic organization and biological properties. These antigens are mainly composed of protein with 6 to 10% carbohydrate. Papain solubilized HL-A antigen molecules have molecular weight of 48,000, H-2 antigens, 40-44,000. The HL-A antigens have a number of antigenic determinants which appear to be common to all HL-A molecules. It has been found that one or more of these HL-A common determinants is carried by an 11,000 fragment that is obtained from HL-A molecules exposed to acid pH or various dissociating reagents. This 11,000 fragment has been shown to be the same as the protein called β_2-microglobulin. This suggests that the HL-A molecules consist of two polypeptide chains, one of 33,000 carrying all the serological specificities and some common determinants and another of 11,000 (β_2-microglobulin) having only the common determinants. Chemical analysis of the large polypeptide chains carrying two different specificities, HL-A$_2$ and HL-A[illegible], is now being carried out and should give some insight into the structural basis for the serological specificities. The dissociation of the common portion fragment (β_2-microglobulin) from the HL-A antigen molecules has usually been found to be accompanied by loss of HL-A alloantigenic activity. It has been stated that the combination of the 33,000 fragment with β_2-microglobulin may well be non-specific. Nevertheless it can be argued that the β_2-microglobulin must exert a function modifying the conformation of the large component to form the original antigenic site. The experiment which would answer this question would be the demonstration of recovery of antigen activity on mixing the two components and this has not yet been demonstrated. Different preparations of β_2-micro-globulins are also being studied in their primary sequence, in order to define some variants that could help in genetic studies.

TUMOR SPECIFIC TRANSPLANTATION ANTIGENS

One of the changes that has been described to be present invariably in cancer cells is the appearance of new antigens. These antigens are associated with the "cancer state" and could be virus antigen, embryonic antigen, tissue specific antigen etc. depending on the type of tumor formed. One class of antigen referred to as tumor specific transplantation antigen (TSTA) occurs on the cell membrane and is detected by classical transplantation rejection procedures in syngeneic hosts. In the oncogenic virus system, the DNA viruses such as polyoma, SV40 and the adeno viruses or the RNA viruses appear to induce TSTAs distinct from virion antigens in inbred strains of mice. The precise role that the TSTA plays in the process of viral oncogenesis has not yet been determined. However, evidence is accumulating that shows that they are capable of evoking immune response of the homograft type that hold in check or eliminate foci of neoplastic cells. The effective immune reaction is cellular in nature and directly related to lymphoid cells. To determine the mechanisms involved for the induction of several types of TSTA the knowledge of the chemical nature of these antigens must be investigated. The aim of our project is the solubilization and purification of two TSTA - one from an RLV(Rauscher virus)-induced leukemia designated RBL-5 and the other an SV40 virus transformed BALB/c line (MKSA). RBL5 cells are immunogenic and provide immunity against other leukemias of the FMR type. RBL5 is also a good target cell and is easily lysed *in vitro* by sensitized syngeneic lymphocytes or by anti-RBL5 antiserum. The method for extraction and partial purification has been described. Principally it involves the use of ascites cells, disruption of the cell by a sonifier or nitrogen bomb, and collection of the membrane by high speed centrifugation at 55,000 g. Pellets obtained were suspended in 0.05M Tris buffer, pH 8.4 and solubilized with papain. The papain solubilized material was concentrated by ultrafiltration and chromatographed on a column of Sephadex G-150. A peak of approximately 50,000 MW containing immunogenic material was recovered. The immunogenic activity recovered here was specific; challenge with a syngeneic tumor No. 89 containing a polyoma virus specific surface antigen did not reveal inhibition of the growth of this neoplasm. This TSTA isolated from RBL5 seems to be of non-virion origin. Infectious virus was not detected on any of the antigen preparations; neutralizing antibodies were not detectable in a syngeneic antiserum. Soluble TSTA has also been obtained from dissociated tumor cells of the ascitic or tissue culture SV40 transformed BALB/c lines (mKSA). These lines are non-virus producing. The methods used have been exactly as reported for the isolation of TSTA from RBL5 or mouse H-2 histocompatibility antigens (2). Immunogenic activity as assayed by tumor rejection was recovered in at least 5% yield and this activity was specific. The solubilized fractions from both RBL5 and mKSA containing the major titers of immunogenic material, chromatographed in a manner similar to what has been reported for the mouse H-2 alloantigens derived from normal lymphoid cells. This suggests that transplantation antigen on a variety of normal or transformed cells are similar in physical-chemical characteristics. Indeed, the approximate molecular weight (50,000) for these antigens has also been reported for a skin sensitizing antigen from rat tumor. Both of the TSTA described here showed good biological activity; however attempts have been made to demonstrate reactivity with specific cytotoxic antisera. With the RBL5 system, by the use of a high titered anti-MSV (MLV) antiserum or antiserum produced in syngeneic mice after repeated immunization with a crude membrane preparation, inhibition of lysis was observed using RBL5 target cells labelled with ^{51}Cr. However, titer decreased drastically as the purification was carried through the chromatography on Sephadex G-150 probably because of lability of the antigen so that the usefulness of this method for monitoring further steps in purification is not ideal. For the mKSA system mKSA-tissue culture cell lines could not react with specific cytotoxic antiserum, although these cells could be lysed by an H2-4 alloantiserum. Other *in vitro* methods are being currently investigated such as microcomplement fixation, anti-immunoglobulin assay and inhibition of leukocyte migration to be used to follow purification of these antigens.

Objective 3: Approach 2, 4, 5

Publications:

Friedenson, B., Appella, E., Takeda, Y., Roholt, O.A., Pressman, D.: Immunoglobulin G antibodies from an individual rabbit in which several heavy chain variants are paired with one light chain sequence. *J. Biol. Chem.* 248: 7073-7079, 1973.

Law, L. W., Appella, E., Cohen, J., Dean, J.: Reactivity of lymphoid cells from mice humorally tolerant to histocompatibility antigens. *Nature New Biol.*, 246: 174-176, 1973.

Drapkin, M. S., Appella, E., Law, L. W.: Immunogenic properties of a soluble tumor specific transplantation antigen induced by Simian virus 40. *J. Nat. Cancer Inst.* 52: 259-264, 1974.

Mage, R. G., Carta-Sorcini, M., Appella, E.: Peptides with a-locus allotypic determinants. *Ann. Immunol. (Inst. Pasteur)*, 125C, 97-18, 1974.

Tanigaki, N., Nakamuro, K., Appella, E., Poulik, M.D., Pressman, D.: Identity of the HL-A common portion fragment and human β_2-microglobulin. *Biochem. Biophys. Res. Comm.*, 55: 1234-1239, 1973.

Zimmerman, C. L., Pisano, J. J., Appella, E.: Analysis of amino acid phenylthiohydantoins by high speed liquid chromatography. *Biochem. Biophys. Res. Comm.*, 55: 1220-1224, 1973.

Appella, E., Chersi, A., Rejnek, J., Reisfeld, R., Mage, R.: Rabbit immunoglobulin lambda chains: Isolation and amino acid sequence of cysteine-containing peptides. *Immunochemistry*, 11, 1974.

Robinson, E. A., Smith, D. F., Appella, E.: Chemical characterization of a mouse immunoglobulin A heavy chain with a 100 residue deletion: Amino acid and carbohydrate compositions and amino- and carboxy-termini sequences. *J. Biol. Chem.*, 1974 (in press).

Serial No. NCI-4833
1. Laboratory of Cell Biology
2. Ultrastructural Biol. Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electron microscope, biological and biochemical studies of normal, malignant and virus infected cells and cell fractions.

Previous Serial Number: Same

Principal Investigator: Dr. Nelson A. Wivel
Dr. Stringner Sue Yang

Other Investigators:
Dr. L. W. Law (NCI-3200)
Dr. D. V. Ablashi (NCI-4834
Dr. G. Smith (NCI-3526)
Dr. E. B. Thompson (NCI-5210)
Dr. P. G. Calarco (Univ. of Calif.)

Man Years:
Total - 3.00
Professional - 2.00
Others - 1.00

Project Description:

Objectives:

The Ultrastructural Biology Section is concerned with the study of various factors which influece cell differentiation and malignant transformation. Particular emphasis is given to systems in which viruses represent the transforming agent. The projects undertaken require the correlation of data from various disciplines such as electron microscopy, virology, biochemistry, and immunology.

Methods Employed:

The principal methods involve the visualization of appropriate selected biological materials in the electron microscope, the use of tissue culture for propagation of viruses, the use of experimental animals for propagation of tumors, the use of differential and density gradient centrifugation to isolate cell and virus fractions, the use of radioisotopes to label viral nucleic acids, and the detection of viral antigens by a variety of immunological techniques.

Major Findings:

1. Intracisternal A particles. We are continuing our ongoing program in the study of intracellular virus-like particles. Our current efforts yielded a further characterization of murine intracisternal A particles. Analysis of the intrinsic RNA-dependent DNA polymerase indicates that it has many similarities to other viral reverse transcriptases. Demonstration of a complete endogenous reaction requires dithiothreitol, Mg^{++}, Na^{+} or K^{+}, and all four deoxyribonucleoside triphosphates. RNase digestion experiments indicated that this DNA polymerase was most probably dependent on the intrinsic A particle RNA as template. The products of the enzyme reaction were synthesized as follows. At the end of 5 minutes the ^{3}H-DNA was clearly linked to the endogenous RNA, but at the end of 30 minutes the product was found to be primarily free DNA. When this DNA was hybridized to the purified RNAs from intracisternal A particles, 69% hybridized to the 70S RNA and 88% to the 35S RNA. Now that a DNA probe is available, we are examining its homology with that of the high molecular weight RNAs of several of the C-type oncornaviruses. Information derived from such experiments will help demonstrate more definitively the genetic relationship of intracisternal A particles to other RNA tumor viruses.

In a collaborative project with Dr. P. Calarco at the Univ. of Calif. we are studying A particles which are replicated during early embryogenesis. It has now been established that these particles are seen in small numbers in dictyate oocytes immediately after their release from ovarian follicles. They were not found in oocytes which reached mataphase II or in fertilized eggs. At the 4 cell stage large numbers of complete particles reappear within the endoplasmic reticulum. By the 16 cell stage and the 32+ cell stage (blastocyst) A particles are only rarely encountered. Current efforts are directed toward comparing the embryogenic A particle antigens with those of A particles from murine tumors, using immuno-electron microscopy.

2. Viral DNA polymerases. Viral DNA polymerases are being studied in a number of different systems. One such system is a simian tissue culture cell line (AA) derived from a lymphoma induced in the owl monkey by Herpesvirus saimiri. H. Saimiri was observed in the monkey's serum only during the first few weeks post inoculation; only viral antigen persisted during the onset of frank leukemia and lymphoma. In the AA lymphoma all line H. Saimiri likewise was not observed and the viral antigen was detectable only up to the 4th passage of the cell culture. Chromosomal aberration occurred during the seventh passage and at the ninth passage there was a spontaneous appearance of a membrane-bound RNA-dependent DNA polymerase which possesses many biochemical properties analogous to those of primate and murine RNA tumor viruses. Characteristics of this reverse transcriptase are clearly distinct from those of the DNA polymerase of H. Saimiri. Since this enzyme activity has features of a viral gene product, its relationship to the primate RNA tumor viruses or to H. Saimiri merits additional investigation

3. Steroid induction of phenylalanine specific transfer RNA in hepatoma tissue culture cells. The phenomenon of hormone-dependent tumor growth has

not yet been well understood. In collaboration with Dr. Brad Thompson, glucocorticoid induction of macromolecular synthesis in hepatoma tissue culture (HTC) cells was studied in vitro and in vivo at both the nucleic acid and protein levels, in an attempt to clarify some of the basic mechanisms of hormone action in tumor growth. It was found that dexamethasone phosphate induced an increase in phenylalanine acceptor activity in vivo. Specifically, such a change was accounted for by an increase in net synthesis of one isoaccepting form of the two known phenylalanine specific transfer RNAs. This phenylalanine tRNA induction by dexamethasone phosphate in HTC cells is coordinated with the induction of tyrosine aminotransferase, a phenomenon described as being under translational control. Thus the action of this steroid on HTC cells resides at the gene level as well as at the translational level.

4. Effect of Hyperimmune Alloanterserum on Cell-Mediated Immunity.

Alloantiserum blocking of cell-mediated immunity by a ^{51}chromium release cell-medicated cytotoxicity assay has been characterized and correlated with immunological enhancement of tumor allografts across H-2 barriers in mice. Cytotoxicity could be blocked by adding alloantiserum directly into the reaction mixture or by pretreating the target cells with alloantiserum. Effector cells could also be specifically blocked by pretreatment with alloantigens or with a fraction isolated from a mixture of alloantiserum and ascitic fluid containing alloantibody, but not with alloantibody alone. Upon purification, this fraction dissociated into IgG and a small molecular species with some biochemical and antigenic properties analogous to H-2 fragments. These observations are consistent with the hypothesis that an antigen-antibody complex consisting of hyperimmune alloantibody and H-2 alloantigen effects blocking of cell-mediated immunity and tumor growth enhancement.

Relationship of research to National Cancer Plan:

Project Area - 10

Approach Elements - 2

Approach - 4

Publications

Yang, S. S., Lippmann, M. and Thompson, E. B.: Transfer ribonucleic acid in hepatoma tissue culture cells. I. Induction in vivo by dexamethasone phosphate of phenylalanine-accepting activity. Endocrinology 94: 254-261, 1974.

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Yang, S. S., Ablashi, D. V., Armstrong, G. and Ting, R. C.: RNA-dependent DNA polymerase associated with a simian lymphoid cell line derived from a Herpesviruse saimiri induced lymphoma. International J. Cancer 13: (1) 82-90, 1974.

Yang, S. S. and Wivel, N. A.: Characterization of an endogenous Rna-dependent DNA polymerase associated with murine intracisternal A particles. J. Virology 13: (3) 1974.

Yang, S. S., Calarco, P. G. and Wivel, N. A.: Biochemical properties and replication of murine intracisternal A-particles during early embryogenesis. The Third Conference on Embryonic and Fetal Antigens in Cancer. Cancer Research 1974. (in press)

Cohen, J. M., Yang, S. S. and Law, L. L.: Abrogation of cell-mediated immunity by hyperimmune alloantiserum: Mechanism and correlation with allograft enhancement. Int. J. Cancer 13: (4) 1974.

Serial No. NCI-5526
1. Macromolecular Biology Section
2. Lab. of Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cell surface changes in normal, DNA virus transformed and spontaneously transformed tumorigenic mouse cell lines.

Previous Serial Number: Same

Principal Investigator: Peter T. Mora

Other Investigators: Dr. Samuel Luborsky, Mrs. V. W. McFarland, Miss Lynda Couvillion, Dr. Samuel Pancake, Dr. Alan Schultz, Guest Worker, MBS, LCB; Dr. Robert Bassin, VLLB, CCP; Dr. John Bader, CH, DCCP, NCI; Dr. Peter Fishman, DMN, NINDS.

Cooperating Units: Dr. P. W. Kent, Director, Glycoprotein Research Unit, University of Durham, England.

Man Years:

Total:	6.2
Professional:	3.2
Other:	3

Project Description:

Objectives: To elucidate certain biochemical changes on the cell surface which occur during transformation to malignancy in mouse cells.

Methods Employed: Tissue culturing methods (humidified CO_2 incubator, roller bottles, suspension cultures, etc.). Labeling of glycolipids and glycoproteins, also of nucleic acids and proteins, etc., with radioactive precursors during cell growth in culture. Cell homogenization and subcellular fractionation techniques such as isolation of cell membranes. Chemical isolation and characterization procedures, including thin-layer chromatography and gel electrophoresis. Analytical and preparative ultracentrifuge studies. Electronmicroscopy. General enzymology techniques. Radioactivity counting procedures. Tumor take studies in mice.

Major Findings: I. Biochemically-induced modification of the cell surface membrane carbohydrates. Accumulating evidence suggests that protein or lipid bound oligosaccharides extending outwards from the plasma membrane of the cells, especially the terminal electronegative sialic acid residues, crucially contribute to various biologic recognition of cell surface structures. Foremost among such situations are the distinctive and well known blood group specifications of human red cells and secretions, where the terminal oligosaccharide structures provide the determinant groups. Current work on other mammalian cells, including human lymphocytes, tumor cells, etc., imply that the terminal carbohydrate residues especially the sialic acids participate in immunologic recognition of the various cells, and also have an important role in cell to cell recognition and in growth control. The external modification of cell surface carbohydrates such as digestion of sialic acids by added neuraminidase is actively pursued in many other laboratories. Under these conditions, however, the striking biological affects upon the binding properties and immunological recognition of such cells are invariably obscured by the prompt resynthesis of this peripherial sugar.

With Dr. Paul W. Kent and subsequently with Dr. Alan Schultz, we have undertaken a systematic study to achieve by internal metabolic means modification of the glycoprotein biosynthesis of the cells, especially that of cell surface sialic acids. Since N-acetyl-glucosamine is a crucial intermediate of the synthesizing enzymes (which include sugar phosphorylating and UDP coupling enzymes) leading to sialic acid, the stereochemically closely related halogenated analogs N-fluoroacetyl-D-glucosamine (Glc NAc F) and the N-iodoacetyl-analogue (Glc NAc I) were obtained from Dr. Kent, and investigated in their effects of interfering with the metabolic utilization of the N-acetyl-glucosamine by normal, spontaneously transformed and virally transformed mouse cells. First, it was determined the limit of concentration of these analogs which was non-toxic to the various cells in culture, by showing that there was no interference in protein, RNA or DNA biosynthesis. Within this non-toxic concentration range, then it was found that the synthesis of glycoproteins as measured by incorporation of labelled glucosamine can be greatly altered: by each halogenated analog the synthesis can be greatly increased, or practically prevented - depending on the dosage and the timing of addition of the halogenated analogs. The analog Glc NAc F was chosen for further careful study this year, to define the molecular (metabolic) basis of the effects when it prevents glycoprotein, especially sialic acid biosynthesis. Dr. Schultz, with the help of various strategically labeled Glc NAc F samples from Dr. Kent's laboratory, proved that the effects of Glc NAc F (at 0.5 mM concentration) was not on modifying various sugars uptake into the cell, but in a striking inhibition of the enzymatic conversion of Glc NAc -6- pH → UDP-Glc NAc, which is the penultimate step required for the synthesis of sialic acid or n-acetyl-neuraminic acid: (→ Man NAc → NANA). The modifier Glc NAc F was found to be metabolized both in a cell free system and in whole cells, which is concordant with the found intervention of the enzymatic process.

Employing fluorinated amino-sugars as probes for, and modifiers of, cell surface glycoprotein constituents offer a new and provocative approach for the systematic control of the information bearing oligosaccharide moiety of the cell surface coat. Of particular importance is the demonstration that these modified amino sugars, which are on the direct pathway of sialic acid biosynthesis, may provide an internal or steady state level of this critical cell determinant. As mentioned above this is a major advance over current techniques which are dependent upon the transitory removal of sialic acid by the addition of exogeneous neuraminidase. An alternate advantage of this system is suggested by the possibility of exploiting the paramagnetic properties of the marker ion (work in collaboration with Dr. Kent) to provide a highly sensitive mechanism for the location and identification of binding sites on the cell membrane.

II. Changes in glycolipid synthesizing enzymes in virally transformed cells. We have decided to carefully evaluate how general our previous finding is (with R. O. Brady) that DNA virus induced transformation, such as SV40 transformation of mouse cells causes a decrease in uridine diphosphate N-acetyl-galactosamine:hematoside N-acetylgalactosaminyl transferase. A carefully cloned cell line was infected with SV40 and recloned (work with L. Couvillion). The T antigen positive clones and the T antigen negative clones were carefully characterized, both in regards of the cellular properties such as growth in tissue culture, SV40 surface antigens detectable by specific serum mediated cytotoxicity assay (with Dr. S. Pancake) and various glycolipid glycosyl transferases including the above N-acetyl-galactosaminyl (Gal NAc) transferase (with Dr. Fishman). All the T antigen positive clones were susceptible to the killing effect of the specific antiserum. However, the glycolipid glycosyl transferase activities, including the N-acetyl-galactosaminyl-transferase revealed no consistent pattern with the T antigen content. Thus we have to conclude that apparently inadvertent and yet unknown, probably selective factors influence the observed changes in glycolipid synthesis after DNA virus induced cell transformation.

With RNA viruses it is possible to transform all the cells in a culture, when the multiplicity of infection is high enough and the appropriate cell -virus combination is being used. Using

Dr. Bassin's S^+ L^- Moloney isolate of murine sarcoma virus Swiss 3T3 cells were transformed quantitatively in two days, as detectable by changes in cell morphology (work with V. W. McFarland). In four days after infection a 50% decrease in Gal NAc transferase and a 3 fold increase in Gal transferase was observed (with Dr. Fishman). Similar changes were observed also on cloned Balb 3T3 cells after transformation with the S+ L^- Moloney sarcoma virus. However, there were no such changes when all the cells were infected with the Moloney leukemia virus.

The decrease in Gal NAc transferase occurs after the morphological transformation induced by the MSV becomes evident. Therefore, the change in morphology of MSV-transformed cells is apparently not a consequence of the decrease in this enzyme level; but it is possible that the decrease in enzyme level is a delayed phenotypic expression of an MSV gene functioning in the transformed cells. Since infection with MLV alone is not associated with a decrease in the enzyme activity at a time when virus is being produced, replication of the entire leukemia virus genome is not sufficient to cause the specific change in N-acetylgalactosaminyltransferase activity. Previous cell transformation by MSV, evidence for functioning of that portion of the sarcoma virus genome which induces transformation in cell morphology, however, appears to be necessary for this change in enzyme activity.

The specific enzyme change - the decrease in Gal NAc transferase, was thus found in two mouse cell systems (Swiss and Balb) transformed by a sarcoma virus. This biochemical change is not due to selection for transformation by the RNA virus of those cells which have inherently low enzyme activity, but more likely is one of the direct but delayed consequences of viral transformation. Only further work will tell how general such phenotypic change is upon various "transformation" of cells in culture. It has to be kept in mind however, that unless the transformation process is 100% and is being followed kinetically, as in our experiments, comparative work on various clonal cell lines derived from each other may well be misleading and may be due to artifacts of cloning and selection. Finally, it is important to remember that "spontaneously" transformed cells which are highly tumorigenic, were shown by us not to have a decrease in Gal NAc transferase activity, or any pattern in any other glycolipid transferase enzyme activity. For these reasons our current research interest is shifting to investigation of cell membrane proteins and glycoproteins.

III. Cell membrane isolation and changes in cell membrane proteins and glycoproteins. The previously mentioned cloned and SV40 infected and recloned cells, with T antigen or without T antigen (these latter cells as controls, both type of cells from

Miss Couvillion) are now being used in experiments to develop cell surface membrane fractionation techniques (with Dr. S. Luborsky and V. McFarland) and to compare the biochemical constitution of the purified membranes (with Dr. S. Pancake). For labelling cell surface membranes metabolically, ^{3}H mannosamine is being used over cultured cells (16 hrs.), followed by a chase to allow the majority of the ^{3}H incorporate into sialic acid, and the ^{3}H glycoproteins to be transported to the cell surface. After gentle hypotonic disruption and sucrose density gradient centrifugation, a surface membrane fraction is being obtained, which is indeed rich in ^{3}H label (Dr. Luborsky). An alternative method employs labelling of the surface proteins of the cells under physiological condition by potassium borotritide. The procedure is based on the interaction of pyridoxal phosphate (an aldehyde which is thought not to penetrate cell membranes) with cell surface amino groups to produce a Schiff base. The Schiff base is then reduced by sodium borotritide resulting in the covalent incorporation of tritium into those cell surface components carrying exposed amino groups. Experiments have been performed under a variety of conditions and with varying reagent concentrations in order to obtain labeled membrane preparations of the high specific activity prequisite to the accomplishment of subsequent analytical analyses while at the same time avoiding excessive labeling of internal cellular components. Our results indicate that this can be best achieved by use of potassium borotritide of a very high specific activity (15 Ci/mmole) at low concentration (70μM) for a limited time period (20 min.) at a slightly alkaline condition (pH 8) at physiological temperature and salt concentration (Dr. Pancake). By this technique too, substantial amount of the labeled material appears in the cell membrane preparations. Current work is in progress in gel electrophoretic resolution of the components of the purified cell membrane preparations, thus comparing both the protein and also the glycoprotein patterns of both the transformed (T antigen positive) and of the control (T antigen negative) clones. It is important to detect in viral transformation whether any of the observed biochemical changes in membranes occur obligatorily or not, and the above analytical work on the carefully matched sister clones is devised for this purpose. The indentification of any difference in membrane composition after viral transformation is of importance, since it may pertain to new surface antigens (TSTA) or to new structures related to other viral function (transformation to tumorigenicity?).

IV. Comparison of SV40 induced antigens on the surface of cultivated cells by a cytolytic microassay (with Dr. Pancake). By means of a direct cytolytic assay, SV40 specific antisera were shown to kill SV40 T antigen positive AL/N mouse cell lines, including lines which were repeatedly passed through the syngeneic mouse as tumors. The sera also killed a polyoma transformed SV40

T antigen negative AL/N cell line, but not untransformed or spontaneously transformed AL/N embryo cell lines. With the antisera tested there was also no lysis observed on SV40 T antigen positive Balb/c mouse, hamster or human cell lines, although by use of a competition assay SV40 specific common antigens were detectable on the surface of these cells. These assays and these type of approaches are being held in abeyance, since the pertinence of this assay to *in vivo* determined transplantation antigen is open to question, at least in a similar SV40 mouse (Balb/c) system.

V. Cell properties after repeated transplantation of spontaneously and of SV40 virus transformed mouse cell lines. (With V. W. McFarland, A. Schultz and S. Luborsky). "Spontaneously" or SV40 virus transformed AL/N mouse cell lines were passed repeatedly through the syngeneic mouse, and derivative cell lines were re-established in culture from minced pieces of tumors in the presence of concentrated fetal calf serum or from the tumor cells dispersed by trypsin. The aim was to compare in the two types of mass cell lines the selection processes which operate during such procedures by characterization of the resulting cell lines. Measurements of the growth in tissue culture on substratum showed no significant differences among any of the cell lines. The SV40 transformed cells and the derivative cells had low anchorage requirement for growth. The greatest anchorage requirement for growth was in the derivative cells from the "spontaneously" transformed cells which were established from minced tumors. The spontaneously transformed cells and all derivative cells had high tumorigenicity *in vivo* ($TD_{50} < 10^2$), and released membrane bound RNA viruses as determined by uridine labeling and isopicnic banding in sucrose density gradient (with Dr. Luborsky). The SV40 transformed cells had no observable tumorigenicity ($TD_{50} > 10^8$) which increased somewhat after transplantations ($1 - 5 \times 10^5$) especially when the cells were tested in irradiated mouse (5×10^4); however, in both immunocompetent and irradiated syngeneic mouse tumor rejection was observed, indicating presence of tumor specific transplantation antigen(s). The SV40 transformed cells did not release RNA virus; the derivative cell lines maintained unaltered their SV40 specific T antigen and their susceptibility to lysis by specific antiserum. There was no consistent pattern in the glycolipid transferases, when newly established cell lines were prepared from the tumors (P. Fishman). Similarly, no consistent pattern of these enzymes was observed in repeat experiments in newly established AL/N embryo cell lines in their third to tenth tissue culture transfer (Couvillion & Fishman).

VI. Spontaneously transformed mouse cell lines become nontumorigenic after transformation by SV40 virus. (With L. Couvillion and S. Pancake). A strongly tumorigenic mouse cell line ($TD_{50} < 100$ cells/mouse) which developed spontaneously in culture from AL/N mouse embryo, was cloned out and was shown to retain its tumorigenicity. This clonal cell line was infected in tissue culture by SV40 virus, and subclones were isolated. Two T antigen positive subclones showed greatly reduced tumorigenicity ($TD_{50} > 10^4$). The other subclones which were T antigen negative were used as control uninfected cells. These subclones had unchanged tumorigenicity, equal to the parent clone. Virus rescue studies and cell surface antigen titrations by in vitro techniques are in progress to show that the T antigen positive clones carry the full virus genome and express cell surface antigens. Comparative biochemical studies on cell surface membrane components, including proteins and glycoproteins are being carried out as described above. Differences have been detected in cell surface antigenic properties between the T antigen positive and negative subclones using the specific serum cytotoxicity assay.

This research shows for the first time that highly tumorigenic cells transformed spontaneously by an unknown process, can be rendered essentially non-tumorigenic by SV40 viral transformation through expression of strong new cell surface antigens.

Newly obtained spontaneously transformed cell lines, their clonal derivatives, and recloned lines after SV40 transformation are under study to define the generality and the obligatoriness of the reversal of cell tumorigenicity by viral transformation.

Significance to Biomedical Research and the Program of the Institute: Studies of cell surface biochemical changes which relate to acquisition of tumorigenic potential in vivo or to immunologic recognition are of interest, especially when they may lead to understanding of changes in molecular mechanism which appears to be reducible to heritable changes on control processes on the DNA level in the virally induced transformation of cells. This work pertains to the National Cancer Plan Objective 3: Develop the means to prevent transformation of normal cells to cells capable of forming cancers; Approach 2: Delineate the nature and rate of oncogenic cell transformation in carcinogenesis (including aspects of cell culture, viruses), and to Approach 5: Study of cell surface and cell membranes.

Proposed Course of the Project: There will be an attempt to further clarify the relevance of the various changes in cell membrane biochemistry (glycoproteins, proteins, etc.) to spontaneous and to viral induced transformation of cells. We also plan to relate these changes, and the changes caused by the amino-sugar biosynthesis modifiers, to phenotypic changes in cell immunogenicity and in tumorigenicity in syngeneic and allogeneic mouse systems. We hope that the information and methodology developed in the mouse system will be of use on human tumor cells.

Honors and Awards: None

Publications:

Kent, P. W. and Mora, P. T.: Modification of glycoprotein biosynthesis in transformed mouse cell lines. In Kulonen, E., and I. Päparaaken (eds.): The Biology of Fibroblasts. New York, N. Y., Academic Press, 1973, pp. 287-301.

Kent, P. W. and Mora, P. T.: Biosynthetically-induced modification of the structure of membrane-bound oligosaccharides. In Kent, P. W. (ed.): Membrane Mediated Information: Vol. I, Biochemical Functions. Oxford and Lancaster, England, Medical Technical Publications, 1973, pp. 130-141.

Mora, P. T.: Cell growth regulation, cell selection and the function of the membrane glycolipids. In Kent, P. W. (ed.): Membrane Mediated Information: Vol. I, Biochemical Functions. Oxford and Lancaster, England, Medical Technical Publications, 1973, pp. 65-84.

Fishman, P. H., Brady, R. O. and Mora, P. T.: Altered glycolipid metabolism related to viral transformation of established mouse cell lines. In Wood, R. (ed.): Cancer Lipids, Biochemistry and Metabolism. American Oil Chemist Society, 1973, pp. 251-266.

Brady, R. O., Fishman, P. H. and Mora, P. T.: Alterations of complex lipid metabolism in tumorigenic virus transformed cell lines. In Weber, G. (ed.): Advances in Enzyme Regulation, Vol. 11. New York, N. Y., Pergamon Press, 1973, pp. 231-251.

Mora, P. T., Fishman, P. H., Bassin, R. H., Brady, R. O. and McFarland, V. W.: Transformation of Swiss 3T3 cells by murine sarcoma virus is followed by decrease in a glycolipid glycosyltransferase. Nature 245: 226-229, 1973.

Pancake, S. J. and Mora, P. T.: Comparison of SV40 induced antigens on the surface of cultivated cells by a cytolytic microassay. Virology, in press.

Serial No. NCI-5540
1. Macromolecular Biology Section
2. Lab. of Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The apparent specific volume of proteins.

Previous Serial Number: Same

Principal Investigator: Samuel W. Luborsky

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 3/8
Professional: 1/8
Other: 1/4

Project Description:

Objectives: As part of a study of the relationship between protein physicochemical properties and activity we wished to measure the apparent specific volume ($\bar{v}$) of a subunit containing protein, asparaginase, and the $\bar{v}$ of a protein which contains covalently bound carbohydrate residues. Knowledge of $\bar{v}$ is essential to the determination of molecular weights from sedimentation equilibrium data. The simplest current alternative method for determination of molecular weights, gel electrophoresis, is influenced by molecular conformation, the presence of covalently linked, non-amino acid residues (e.g., carbohydrate), or bound ligands (e.g., cofactors or detergents). Such results thus depend upon the assurance of purity of sample and its similarity to standards against which it is measured. This latter requirement is often particularly difficult to meet when one wishes to characterize a new protein. Sedimentation equilibrium experiments are not subject to these limitations and give accurate molecular weights if $\bar{v}$ is known for the sample under the conditions of interest. The $\bar{v}$ is calcualted from precise measurements of solution densities.

Methods Employed: Measurements of protein solution densities, using a quartz oscillator densimeter, analytical ultracentrifugation ultraviolet absorption spectroscopy.

Major Findings: To obtain protein $\bar{v}$ with precision near ±0.5% solution density measurements approaching ± $1/10^6$ are required. This necessitates temperature control of the densimeter to better than ±0.01°C, at 20.0°C. The large waterbath and controls set up for this purpose required the design and installation of a measuring thermister circuit which indicates temperature fluctuations on a chart paper. Calibration of thermister and chart scale against an NBS calibrated thermometer indicated that temperature control was about ±.005°C. Solution densities must be obtained by calibration of the densimeter with solutions of known density. To facilitate interpolation of such densities to $\pm 1/10^6$, a fourth power polynomial equation was fitted to data for density vs concentration of KCl solutions (International Critical Tables). As an internal test, it was found that densities of KCl solutions could be obtained to $\pm 5/10^6$, using similar KCl solutions as standards, if the temperature control was better than ±.01°C during the course of the day's measurements.

The density was measured of a series of asparaginase solutions of known absorbance at 278nm. Concentration was obtained from dry weight determination on a solution of known absorbance, from which the extinction, $E^{1\%}_{278} = 7.16 \pm .1$, was obtained. A linear plot of density vs concentration was obtained (slope 0.2803), from which $\bar{v}$ was calculated to be 0.717 ± .009.

The molecular weight of an immunoglobulin fragment (obtained from Dr. E. Appella) was determined by sedimentation equilibrium experiments in the analytical ultracentrifuge equipped with absorption-scanner optics. For this material, the concentration distribution at sedimentation equilibrium reflects the contribution of the carbohydrate residues which are a part of this molecule. On the other hand, $\bar{v}$ calculated from the amino acid composition ignores their contribution. A molecular weight of 36,960 ± 3000 was obtained from measurements in 5M guanidine hydrochloride, using a calculated $\bar{v}$ of 0.725. From the known amino acid and carbohydrate composition, the molecular weight can be estimated to be 44,495. An experimentally determined value for $\bar{v}$ should improve the validity of the measured molecular weight. To measure $\bar{v}$ of an immunoglobulin fragment in 5M guanidine hydrochloride, standard KCl solutions were prepared at appropriate densities. Since these concentrated solutions evaporate readily, aliquots of the standards and of the guanidine hydrochloride solvent, against which the samples had been dialyzed, were stored in closed flask and opened only at the time of measurement, and then discarded. Density was measured at a series of concentrations, by recovering and diluting samples immediately following measurement. Concentrations were determined from the absorbance at 280nm, together with a dry weight determination, from which the extinction, $E^{1\%}_{280} = 7.91$. The densities obtained in this manner however were not consistent, even for repeated measurements on a given sample on successive days; so that no useful $\bar{v}$ could be calculated. It was clear from measurements on successive guanidine hydrochloride solutions that sufficient evaporation had occurred among the closed, stored buffer aliquots to significantly alter their densities and reduce agreement to about $1/10^4$.

Significance to Biomedical Research and the Program of the Institute: Studies of the properties of proteins, immunoglobulins and of cell surface membrane fragments now are often carried out by gel electrophoresis, from which molecular weights are frequently estimated. Because of conformational effects these are often found to be in error when later compositional analysis is completed. Knowledge of the effect of the non-amino acid residues upon $\bar{v}$ should provide a better basis to determine accurate molecular weights by conventional sedimentation equilibrium analysis. Extent of protein aggregation is often a basis of cellular control mechanisms. Since asparaginase in its tetrameric form possesses significant anti-lymphoma activity, study of its properties may help one to enhance this activity or to reduce its undesirable side effects (see Project No. NCI-5543). Thus studies of the stability of such interactions are important to an understanding of such mechanisms. This project pertains to the National Cancer Plan Objective 6, Approach 2. Discover new therapeutic agents and develop means for their selection.

Proposed Course of the Project: Measurement of $\bar{v}$ of protein will be held temporarily in abeyance, since our efforts now will be concentrated first for isolating cell membrane proteins and glycoproteins from normal and transformed cells (Project No. NCI-5544). After isolating and purifying proteins or glycoproteins which are different in the normal cell membranes from transformed cell membranes, the characterization techniques may include the physical measurements developed in this project.

Honors and Awards: None

Publications: None

Serial No. NCI-5543
1. Lab. of Cell Biology
2. Macromolecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Physicochemical properties and molecular architecture of L-asparaginases.

Previous Serial Number: None

Principal Investigator: Sidney Shifrin

Other Investigators: Samuel Luborsky, NCI, Stuart Rudikoff, NCI and Joseph Roberts

Cooperating Unit: Sloan-Kettering Institute for Cancer Research

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives: The purpose of this project is to determine the amino acid side chains of L-asparaginase from various bacterial sources that function in binding the substrate and that are required for the maintenance of the enzymatically active enzyme.

Methods Employed: Ultraviolet absorption spectroscopy, difference spectroscopy, fluorescence spectroscopy, circular dichroism, sedimentation velocity ultracentriufgation, polyacrylamide disc gel electrophoresis, chemical modification of amino acid side chains, amino acid analysis and determination of amino acid sequence.

Major Findings: a. D-Asparagine occupies the same substrate binding site as the L-isomer and protects the tetrameric enzyme from the denaturing action of urea. A study of the rate of dissociation of the oligomer as a function of pH indicates that functional groups with a pK of 4.2 and 8.8 participate in binding the substrate. The γ-carboxyl group of a glutamyl residue has a pK of 4.2 while the α-amino group of asparagine has a pK of 8.8. Chemical modification of a tyrosyl residue of the enzyme indicates that the hydroxyl group of this aromatic amino acid is also involved in substrate binding.

b. During the course of renaturation of denatured L-asparaginase, the individual polypeptide chain rapidly refolds into its native conformation. There is a slow process of the reassociation of the individual monomers into the active tetramer. The rate of reassembly is accelerated in the presence of D- and L-asparagine, partially accelerated by D- and L-aspartic acid, and is unaffected by L- or D-glutamine.

c. The addition of glycerol, ethylene glycol and other low molecular weight polyalcohols enhances the rate of dissociation of the tetramer. Higher concentrations of these small non-aqueous solvents of low concentrations of larger hydrophilic solutes protect the quaternary structure of the protein. These solutes modify the nature of the structured water surrounding proteins and enhance the strength of hydrophobic interactions.

d. The similarities in amino acid sequences of the N-terminal region of L-asparaginases purified from Escherichia coli, Erwinia carotovora and the glutaminase-asparaginase from Acinetobacter are unexpected particularly since these residues are not located at the catalytically active site. This suggests that there was a considerable amount of conservation of this enzyme during the course of evolution.

e. Extensive succinylation of the lysyl residues of the glutaminase-asparaginases from Acinetobacter and from Pseudomonas does not result in dissociation into subunits as had been observed with the enzyme from Escherichia coli. Also the catalytic activity of the enzyme is not altered during the course of chemical modification. This chemically modified protein is being examined for its effect on a variety of animal tumors.

Significance to Biomedical Research and the Program of the Institute: An elucidation of the structural features of L-asparaginases from a variety of sources should give a more rational basis for the use of this enzyme as a chemotherapeutic agent in the treatment of cancer. Modification of the isoelectric point may influence the entry of this enzyme into various tissues thereby permitting it to be clinically effective.

Proposed Course of the Project: Examination of the structure of proteins capable of hydrolyzing L-asparagine will continue to be examined in order to ascertain the molecular features which determine the chemotherapeutic value of the enzyme and to chemically modify the enzymes so that their catalytic activity is enhanced and they can be transported more easily into a variety of tumors.

Honors and Awards: None

Publications:

Shifrin, S., Solis, B. G., and Chaiken, I. M.: L-Asparaginase from *Erwinia carotovora*. Physicochemical properties of the native and succinylated enzyme. *J. Biol. Chem.* 248: 3464-3469, 1973.

Shifrin, S., Parrott, C. L., and Luborsky, S. W.: Substrate binding and intersubunit interactions in L-asparaginase. *J. Biol. Chem.* 249: 1335, 1974.

Shifrin, S., and Parrott, C. L.: *In vitro* assembly of L-Asparaginase subunits. *J. Biol. Chem.* 249: June, 1974.

Shifrin, S.: The role of charge transfer in pharmacology. In Cavallito, C. J. (ed.): *Structure Activity Relationships*. New York, N. Y., Pergamon Press, 1973, pp. 167-224.

Serial No. NCI-5544
1. Macromolecular Biology Section
2. Lab. of Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Isolation and comparison of cell membranes of normal and transformed cells.

Previous Serial Number: None

Principal Investigator: Samuel W. Luborsky

Other Investigators: Peter T. Mora and Samuel J. Pancake, LCB,DCBD,NCI

Cooperating Units: None

Man Years:

Total:	1 5/8
Professional:	7/8
Other:	3/4

Project Description:

Objectives: As part of a study of surface changes in transformed cells we wished to develop methods to isolate cell surface membranes to compare those isolated from cloned transformed mouse embryo cells with those from untransformed, cloned, sister cell lines.

Methods Employed: Tissue culture cell cultivation, infection with SV40 virus, cell cloning procedures, standard biochemical techniques, polyacrylamide gel electrophoresis.

Major Findings: Based on techniques reported in the literature a method was developed to isolate the surface membrane fraction from a cell homogenate. After allowing the cells to swell in a hypotonic medium, they are broken in a Dounce homogenizer, and the membrane fraction collected on a sucrose concentration interface, between 30 and 45% (w/w). Protein determination by the method of Lowry gave a measure of total protein recovered. Cells are labelled by adding 2 μc/ml ^{3}H-mannosamine to the growth medium for 24 hours, then allowing an additional "chase" period of 15-24 hours in unlabelled medium before harvesting. The ^{3}H-mannosamine labels specifically the sialic acid residues of the membrane glycoproteins and provides a simple assay for the presence of surface membrane. The isolation procedure was also applied to cells which had been chemically labelled by reaction with pyridoxal phosphate and sodium borotritide, a reaction supposed to label predominantly the surface of the cells (with S. Pancake). The specific activities obtained using the sugar label were approximately 20,000 cpm/mg protein, while the borotritide procedure yielded 10-100 X higher specific activities, suitable for seeking differences in protein composition by disc gel electrophoresis. These experiments are in progress.

The cell lines tested are continuous culture lines derived from AL/N mouse fibroblasts and carried in our laboratory. Some of these lines have become spontaneously transformed, others have been transformed by infection with SV40 virus. The lines were cloned both before and after treatment with SV40 virus, and a series of SV40 T antigen positive and negative cloned lines are available. The membrane isolation procedure was developed using one of the T antigen positive lines, clone-1, and has been applied as well to T antigen negative line, clone-5, for which only preliminary results are available now.

Significance to Biomedical Research and the Program of the Institute: Cell growth characteristics and the ability of certain cells to form tumors in animals depend upon a complex set of interactions between the cell and its host, many aspects of which are thought to be affected by the properties of the cell surface, its chemical composition, structure, and antigenic determinants. These properties are only poorly understood. Methods, such as those outlined here, must be developed for the reproducible isolation of cell membranes for further study. Any differences in the biochemistry of the membranes from normal, "spontantously" transformed, and virally transformed cells should provide important clues to a better understanding of these different states, and potentially provide means for better immunological control of cancer. This project pertains to the National Cancer Plan Objective 3: Develop the means to prevent transformation of normal cells capable of forming cancer; Approach 2: Delineate the nature and rate of oncogenic cell transformations in carcinogenesis (include aspects of cell culture, viruses); and Approach 5: Study of cell surface and cell membranes.

Proposed Course of the Project: Different labelling procedures for the cell surface membrane will be compared, both before and after treatment of the labelled cells with trypsin or neuraminidase, to further test the specificity of these labelling reactions. Other normal, spontaneously transformed, and SV40 infected T antigen positive and negative sister clone lines will be examined.

The nature of membrane compositional differences will be analyzed by polyacrylamide disc gel electrophoresis including radioautography of separated labelled components. Our aim will be to seek a consistent common protein and/or glycoprotein change among many cloned cell lines having a common history and differing only in their possession of the SV40 T antigen, and to seek to correlate any such differences with other biological properties of the cells, such as their antigenicity and their tumorigenicity.

Honors and Awards: None

Publications: None

Serial No. NCI-5550
1. Laboratory of Cell Biology
2. Viral Oncogenesis Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Oncogenic RNA Viruses

Previous Serial Number: Same

Principal Investigator: Kenneth S. S. Chang, M.D., Sc.D.

Other Investigator: Dr. L. W. Law (C:LCB-3200)

Cooperating Units: Immunology Section, Viral Leukemia and Lymphoma Branch, NCI

Man Years: Total: 2.5
Professional: 1.00
Other: 1.5

Project Description:

I. Virological and immunological studies of type C RNA viruses (D1-MuLV) isolated from Hodgkin's like reticulum cell neoplasms of SJL/J mice

The SJL/J strain of mouse which manifests a high incidence of spontaneous Hodgkin's like reticulum cell neoplasm, type-B (RCN-B) was studied to elucidate the possible role played by type-C RNA viruses in the pathogenesis of RCN-B in general. It was found that the virus, designated D1-MuLV, which was isolated from a mouse bearing RCN-B, showed unique type-specific envelope antigens. By in vitro neutralization tests and immunoelectron microscopy, the viral envelope antigen (VEA) of this virus was shown to be distinct from the VEA of a number of different murine type-C viruses, including Gross, Friend, Moloney, Rauscher murine leukemia viruses (MuLV), or the MuLV associated with murine plasmacytomas and BALB/3T3 S2 (spontaneously transformed) cells. However, a cross-reacting VEA was found on a subpopulation of AKR-MuLV and a radiation-induced leukemia virus of A strain mice. The antigen of D1 tumor, a lymphoblastic leukemia induced in SJL/J mice by neonatal inoculation of D1-MuLV, was also shown to be unique in that none of the virus-coded membrane antigens of the cell, such as GCSA or FMR, or alloantigens PC1 and G_{ix} associated with MuLV infection was present on the D1 cell surface.

These findings contributed to the development of an important concept that there existed in various mouse strains a wide variety of type-C viruses antigenically different from each other. This concept is in contrast to the commonly held view that all type-C viruses spontaneously arising in murine tissues and tumors are the same or variants of Gross-MuLV.

Pathogenicity studies of D1-MuLV also indicated some distinctive properties of this virus. When inoculated into neonatal SJL/J and AL/N mice, lymphoblastic leukemias were induced. Further studies indicated that RCN-B was induced in high frequency (30%) in BALB/c mice that had been neonatally thymectomized and inoculated with D1-MuLV. An unexpected finding in this experiment was that a large amount of an MuLV with B-tropism and envelope antigens different from those of D1-MuLV was recovered from the neoplastic tissues of those mice that developed RCN-B in 5 months after inoculation. Thus a possible activation of the endogenous type-C virus is strongly suggested. It is important to note that young BALB/c mice under 6 months of age show very low frequency of infectious type-C virus in their tissues, and that the virus recovered was B-tropic rather than N-tropic, since most of the virus isolates from BALB/c mice under 12 months of age were N-tropic. Such an activation of covert viral genome in vivo by infection with another virus of different tropism opens a new possibility of elucidating viral oncogenesis.

Significance for Cancer Research (NCP Objective 3 Approach 2)

These studies will define the role of type-C RNA viruses in oncogenesis of RCN-B in mice which has some histopathologic resemblance to human Hodgkin's disease. The diversity in viral envelope antigens, cellular membrane antigens coded for by these viruses, and biologic characteristics such as N- and B-tropism, and the pathogenicity in mice may be of significance for understanding of co-evolution of viral and host cell genomes, and for elucidation of processes of viral oncogenesis.

II. Characterization of Solubilized Tumor Antigens Isolated from an RNA Virus-transformed Neoplasm

Previous studies on solubilization and partial purification of histocompatibility antigens (H-2) of mice provided a model for the present study. In cooperation with Drs. Law and Appella, tumor antigens were solubilized and isolated from cell membranes of RBL-5 leukemia cells, and characterized by virological and immunological techniques.

The neoplasm, RBL-5, arose in a C57BL/6 mouse infected with Rauscher leukemia virus (RLV), and was carried in ascitic form. The soluble antigens were prepared by disruption, sonication of cells, differential centrifugation, followed by partial papain digestion of the crude membrane preparation and fractionation by passing through Sephadex G150 column, yielding 3 fractions F1, F2, and F3.

Two weekly s.c. injections of 400 ug crude membrane preparation, or 100 ug F2 fraction, but not F1 or F3 fractions, each mixed with complete Freund's adjuvant, were found to effectively protect the mice against i.p. challenge of a Friend virus induced lymphocytic leukemia (FBL-3) which shared a common antigen with RBL-5. Similar experiments with LSTRA, another Rauscher leukemia virus induced lymphocytic leukemia in (BALB/c x BL/6) F1 mice gave comparable results. FBL-3 and LSTRA were used in this system because they were more immunosensitive than RBL-5 in vivo.

One of the problems encounted in this system was to distinguish whether or not these surface antigens are virus structural proteins or are of non-virion origin but nevertheless coded by the leukemia virus. To detect infectious type-C viruses possibly present in these preparations, NIH Swiss mouse embryo cells treated with DEAE-dextran were inoculated, and after 8 days of cultivation, were cocultivated with XC cells, which acted as an indicator of infectious type-C viruses as shown by formation of syncytial cells. None of these preparations showed any infectious type-C viruses.

The syngeneic antiserum prepared in BL/6 mice by immunization with a mixture of crude membrane or of crude soluble and complete Freund's adjuvant did not manifest any neutralization antibody activity against RLV or MSV(RLV) by XC plaque forming units or focus forming unit reduction assay on NIH mouse embryo cell cultures. This would indicate that the crude membrane used for immunization contained little virion antigens capable of inducing detectable amounts of neutralizing antibody.

A micro-complement fixation test was performed on the RBL-5 derived antigen preparations as well as on high titered RLV and MLV (Moloney leukemia virus) stocks which were prepared from mouse embryo cell culture supernates. These antigens were tested against broad-reacting antisera, such as Fischer rat anti-MSV(MLV) and hamster anti-RLV sera, which reacted with viral gs as well as envelope antigens. All antigen preparations except F1 showed the presence of virion antigens. The minimal concentrations in terms of protein ug/ml of antigens detectable by this method were 62-250 ug/ml. Two lots of syngeneic anti-crude membrane serum tested also reacted with these preparations with various degrees. It is interesting to note that the minimal concentration of F2 antigen detectable by these syngeneic antisera was very low, i.e. ≤1.8 ug/ml, whereas the minimal detectable antigen concentrations of other preparations were not so low. Such differential CF reactivity would suggest that the syngenic antisera were detecting non-virion antigens besides virion antigens. Another lot of syngeneic antiserum showed a reactivity with crude soluble and F2 antigens without at the same time showing any reactivity with RLV or MLV. This would appear to indicate that the syngeneic antiserum contained an antibody which reacted exclusively with non-virion antigen which is present in crude soluble preparation and partially purified and concentrated in F2 preparation.

The immunological specificities of the activity of these RBL-5 antigens were indicated by the failure of the crude-membrane-immunized mice to be protected against the challenge by polyoma-specific neoplasm and by the failure of Gross virus induced syngeneic leukemic cells, E♂G2 to absorb from the syngeneic anti-RBL-5-crude membrane serum the CF antibody reacting with F2 antigen.

Further, the F2 preparation was the only fraction that contained a blocking activity against the complement-dependent cytotoxicity reaction of the syngeneic anti-RBL-5 crude membrane serum as assayed by Cr^{51}-release, or trypan blue staining techniques.

We conclude from these findings that a molecular entity with the characteristics of a tumor antigen of the transplantation type has been solubilized from a crude membrane preparation of RBL-5 cells, an RNA virus-transformed neoplasm. We now have a sensitive, simple, and reproducible *in vitro* technique to monitor the tumor antigen for further purification and characterization.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Among tumor-associated membrane antigens found on neoplastic cells, the tumor antigens of the transplantation type are of particular interest since they may be used to advantage in acting as immunological surveillance factors, or vaccines, in the prevention of neoplasms, or as immnotherapeutic agents. Any of the tumor antigens identified on the cell surface could interfere with membrane-located growth controlling mechanisms. It is therefore obvious that solubilization and purification of these antigens would provide a major step toward understanding the mechanisms of carcinogenesis and growth controlling events.

Publications:

1. Chang, K. S. S., Law, L. W., and Aoki, T.: Type-C RNA virus isolated from SJL/J mice. *J. Nat. Cancer Inst*. 52: 777-784, 1974.

2. Aoki, T., Huebner, R. J., Chang, K. S. S., Sturm, M. M., and Liu, M.: Diversity of envelope antigens on murine type-C viruses. *J. Nat. Cancer Inst*. 52: 1189-1197, 1974.

3. Aoki, T. and Chang, K. S. S.: Type-C RNA viruses: Diverse immunological and etiological roles. In Bernard, S. (ed.): *Advances in the Biosciences* Braunschweig, Germany, Pergamon Press, 1974, (in press).

4. Law, L. W., Chang, K. S. S., and Nakata, K.: Heterogeneity of virus production and antigenicity detected in cell clones derived from a "non-producer" neoplasm induced by Moloney murine sarcoma virus. *J. Nat. Cancer Inst*. 52: 437-443, 1974.

Serial No. NCI-5552
1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Production and analysis of hybrid somatic cells: their application to some problems of importance in biology and medicine.

Previous Serial Number: Same

Principal Investigator: Hayden G. Coon, Ph.D.

Other Investigators:
Dr. Igor B. Dawid, Carnegie Inst. of Wash, Baltimore.
Dr. William Upholt, Carnegie Inst. of Wash, Baltimore.
Dr. G. David Trisler (C:LCB)
Dr. Premkummar Reddy (C:LCB)
Dr. Jay Robbins and Dr. Kenneth Kraemer (C:D)
Dr. H. V. Gelboin and Dr. J. Whitlock (C:CH)
Dr. Marshall Nirenberg and Dr. Alan Peterkofsky (H:LBG)

Cooperating Units: None

Man Years:
Total: 2.5
Professional: 1.00
Other: 1.5

Project Description:

A) Introduction

Our unit has been doing research aimed at learning how to manipulate the various forms of genetic material in vertebrate cells and the development of systems in which ordinary genetic manipulations such as mutation, recombination, etc., can be practiced with the somatic cells of vertebrate tissues. It is obvious that a more complete ability to manipulate the genetics of vertebrate cells must precede and will probably prove to be the means by which we shall come to understand the biochemical mechanisms at work in normal and malignant cells.

A new area of research, a multiple specialty, has emerged in the past ten years which is now called "Somatic Cell Genetics" or simply, "Cellular Genetics." The production, husbandry, and analysis of hybrid cells are vital components of this new field. The areas of expertise required are: 1) sophisticated animal cell culture; 2) modern methodology for analysis of chromosome morphology and chemistry; 3) biochemical analysis - our work has consisted of assays of enzyme activities, purification and identification of certain protein products, e.g., immunoglobulin and collagen, and the detection of characteristic nucleotide sequences by DNA-DNA and RNA-DNA molecular hybridization. Immunochemical techniques have become increasingly important in our work especially in studying antigens specific to the nervous system that are expressed in hybrids of fibroblasts and nerve cells. It is the combination of these multiple specialties into a single laboratory working on a common group of problems centering upon the unique genetic material found in hybrid cells which is the main promise of the new field in attempting to understand cellular mechanisms in normal and malignant cells.

B) Achievements in specific project areas:

1) During this year a breakthrough has occurred in our effort to produce replicating cell strains from non-dividing cells such as normal neurons, plasma cells, and heart muscle by means of hybridization with dividing cell lines. The original rationale of this kind of experiment was to test the notion that the negative growth controlling elements of normal postmitotic cells might be defeated by fusion with a dividing cell. Henry Harris and his coworkers had demonstrated in another kind of system, the reactivation of chick red cell nuclei in Ehrlich ascites tumor cells, that DNA synthesis and ultimately cell division would occur in the heterokaryon involving both chick and mouse nuclei. The implications for cancer research were obvious, cell fusion in vivo might turn out to be an important mechanism in carcinogenesis, and all methods for manipulating the growth controlling mechanisms of normal cells should be explored. By studying the exconjugants from such hybrid strains (after complete chromosome segregation had occurred) the individual human chromosomes responsible for defeating the normal cells' growth control mechanism might be found. [An extensive study by Henry Harris and George Klein and a preliminary sort of effort by Leo Sachs had already been undertaken to search for the chromosomes responsible for maintenance of malignancy using segregating hybrid cells.] Our theory was that by fusing a mouse neuron with a human fibroblast a replicating hybrid would be formed which would be expected to lose human chromosomes and become more and more like the neuron parent. To our dismay, over 95% of such hybrids made between freshly isolated rodent cells when fused with a human cell line made strains which lost rodent chromosomes: reverse segregants. While this discovery had other uses and other ramifications, our original aims were effectively frustrated.

An attempt was made to discover just when "freshly isolated" rodent cells became "tissue culture" cells and might then be expected to yield hybrids which segregated human chromosomes as all previously known human-rodent (tissue culture cell) hybrids had done. It was learned that Chinese hamster and mouse neurons require a period of adaptation to culture of from 5 to 10 days, after which time they might be expected to make normally segregating hybrids with the human cell lines. The only feasible way of doing this with neurons is to co-cultivate them in non-selective medium with the human cell strain to which they will be fused in 5 to 10 days time. It is crucial that the neuron suspensions first be prepurified using the overnight culture period we have described previously, otherwise contaminating fibroblast-like cells present in the initial suspensions would be expected to overgrow the cultures and then efficiently hybridize thus beclouding the issue for neuron hybrids. The hybrid strains produced are indeed finally losing human chromosomes. They were first produced in August and September and will soon be ready for neurochemical investigation by Nirenberg's group. Our experiments with these cells have been confined to preliminary testing for acetylcholine esterase, process formation, and their use to elicit antibodies with confined specificity to the embryonic nervous system. The important point for the present is that we have finally learned to do what we have struggled three years to accomplish: produce replicating hybrid cells between normal rodent cells and human cell lines which lose human chromosomes.

2) The heterodox segregation of rodent chromosomes in our original series of freshly isolated rodent neuron by human fibroblast cell line hybrids has begun to yield to the very slow and tedious process of chromosome analysis by quinacrine mustard banding. We have succeeded (thanks to about 750 hours donated by members of the senior investigator's family) in analysing approximately 50 metaphases from human-mouse hybrid cells which contained recombinant mt DNA. These cells have been analysed so that each chromosome can be identified individually in each of the metaphases (15-80 mouse plus 76 human chromosomes). The first two cell lines thus examined have yielded a surprising discovery. The reverse segregant human-mouse hybrid lines appear to lose mouse chromosomes until they have a nearly perfect haploid mouse complement remaining with the hypotetraploid complement of the human cell line. This haploid-mouse/heteroploid human cell's lineage then acts as a stem cell which is maintained in the population at about 20% of the total numbers. The remaining 80% of the cells in the population represent rapidly segregating sublines of hybrid cells which appear to be losing the mouse chromosomes at random with all possible combinations (nearly 20!) present in some cells of the population at one time or another. Attempts to clone these populations yield two kinds of colonies - those with very few or no mouse chromosomes (presumably descendants of the segregated cells in the parent population) and colonies with many mouse chromosomes including the "stem cells" (presumably the descendants of the stem cell class in the parent population). It is apparent that the true segregants, those cell lineages which have lost chromosomes from the haploid mouse complement, do not enjoy any selective advantage in the hybrid population. As soon as we can accumulate enough fully analysed metaphases we can run a computer simulation in order to fit our data to various birth-death kinetic models. These observations will probably have important bearing on the use of hybrid cell strains for mapping and virus host range studies and perhaps on the eventual understanding of the processes of chromosome loss in hybrid cells.

Naturally any study which could take advantage of a nearly perfect haploid mouse cell line (albeit together with a flock of human chromosomes) would be able to benefit immediately from using our cell lines.

3) In collaboration with John Minna (H:LBG) 20 isozymes (which can be distinguished from mouse and man) have been assayed by starch gel electro-

phoresis in each of 25 of the reverse segregant human-mouse hybrids. In addition to confirming reverse segregation of mouse genes, evidence has been reported that HGPRTase, GPI, and PGK are syntenic in the mouse X chromosome as they are in the human X chromosome. This work has been submitted to Nature.

4) Ms Ann E. Rahe has succeeded in isolating the first BUdR resistant avian cell line - it is HAT sensitive and presumably is deficient for the enzyme thymidine kinase. The mutant was isolated from our original line of goose sternal fibroblasts, CGBQ-Cl, after ethylmethane sulfonate treatment and several rounds of BUdR-photolysis selection. These mutant avian cell lines will make possible for the first time a detailed study of inter-specific hybrids of birds (and their tumor viruses) as well as the first inter-class hybrids between avian and mammalian cells. These latter should be of great help in producing replicating haploid mammalian cell strains because of the high degree of incompatibility between mammalian and avian chromosomes.

5) Dr. G. David Trisler, whose doctoral dissertation was completed during this reporting period (Indiana University), has described the first set of hybrids between avian erythrocytes and several mammalian cell strains which have been found to retain more than one gene from the avian parent. Dr. Trisler, who has applied for an NIH post doctoral fellowship, is presently making antisera to the new series of normal segregant neuron-fibroblast hybrids and testing the specificities of these antisera and their use as probes for normal neuronal antigens using indirect immunofluorescence on dissected regions of the rodent nervous system.

6) Finally, a new problem, undertaken in collaboration with Dr. Premkummar Reddy (C:LCB), seeks to take advantage of our expertise in chromosome identification by quinacrine mustard fluorescence banding to identify the chromosomal locus in mouse and man of the heavy chain genes by in situ hybridization with his preparations of immunoglobulin heavy chain mRNA.

C) Completed projects and collaborations:

Several projects described in previous years' annual reports were completed (and the collaborations terminated) during the present reporting year.

The collaboration with Jay Robbins and Ken Kraemer (C:D) of the Dermatology Branch concerning the several genetic forms of xeroderma pigmentosum as revealed by complementation in heterokaryons has been concluded. One paper has appeared, one is in preparation and another will be forthcoming.

The collaboration with John Minna (H:BG) on reverse segregant human-mouse hybrids has been substantially completed and three publications have resulted, two in press and one still in preparation. Our collaboration with the Laboratory of Molecular Genetics, NHLI, however, will continue for the neurochemistry of our neuron-fibroblast hybrid cells with Marshall Nirenberg and Alan Peterkofsky and post doctoral fellows in their groups.

Publications:

Wiebel, F. J., Gelboin, H. V., and Coon, H. G.: Regulation of aryl hydrocarbon hydroxylase in intraspecific hybrids of human, mouse and hamster cells. *Proc. Nat. Acad. Sci*. 69: 3580-3584, 1972.

Nyormoi, O., Coon, H. G. and Sinclair, J. H.: Proliferating hybrid cells formed between rat spermatids and an established line of mouse fibroblasts. *J. of Cell Sci*. 13: 863, 1973.

Coon, H. G., Horak, I., and Dawid, I. B.: Propagation of both parental mitochondrial DNAs in rat-human and mouse-human hybrid cells. *J. Mol. Biol*. 81: 285-298, 1973.

Coon, H. G., Horak, I., and Dawid, I. B.: The fate of the mitochondrial DNAs in mouse-human and rat-human hybrid cells. In Davidson, R. L. and De La Cruz, F. F. (eds.): *Somatic Cell Hybridization* . New York, N. Y., Raven Press, 1974.

Horak, I., Dawid, I. B., and Coon, H. G.: Recombination of mitochondrial DNA in somatic hybrid cells. In Carnegie Inst. of Wash., Dept. of Embryology: *Carnegie Institution Year Book*. Baltimore, Md., Garamond Pridemark Press, 1973, pp. 40-42.

Dawid, I. B., Horak, I., and Coon, H. G.: The use of hybrid somatic cells as an approach to mitochondrial genetics in animals. Proc. of the XIII Int. Cong. Genetics. *Suppl. Genetics*, 1974. In press.

Minna, J. D., Yavalow, J., and Coon, H. G.: Expression of phenotypes in hybrid somatic cells derived from the nervous system. Proc. of the XIII Int. Cong. of Genetics. *Suppl. Genetics*, 1974. In press.

Horak, I., Coon, H. G., and Dawid, I. B.: Interspecific recombination of mitochondrial DNA molecules in hybrid somatic cells. *Proc. Natl. Acad. Sci. USA*, 1974. In press.

Robbins, J. A., Kraemer, K. L., Lutzner, M. A., Festoff, B. W., and Coon, H. G.: Xeroderma pigmentosum - an inherited disease with sun sensitivity, multiple cutaneous neoplasms, and abnormal DNA repair. *Ann. Intern. Med*. 80: 221-248, 1974.

Dawid, I. B., Horak, I., and Coon, H. G.: Propagation and recombination of parental mtDNAs in hybrid cells. *International Conference on the Biogenesis of Mitochondria*. New York, N. Y., Academic Press, 1974. In press.

Serial No. NCI-5553
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Structure of mouse myeloma proteins with antibody activity

Previous Serial Number: Same

Principal Investigator: Dr. Stuart Rudikoff

Other Investigators:
Dr. Michael Potter (NCI-5556)
Dr. Eduardo Padlan (NIAMDD-LMB-1)
Dr. David Segal (NIAMDD-LMB-1)
Dr. David Davies (NIAMDD-LMB-1)
Dr. Neil Glaudemans (NIAMDD)
Dr. Leroy Hood (Cal. Tech.)

Man Years:

Total: 2.00
Professional: 1.00
Others: 1.00

Project Description:

Plasmacytomas which produce homogeneous immunoglobulins have been induced in the inbred BALB/c strain of mouse by Dr. Michael Potter. Many of these homogeneous immunoglobulins have been shown to demonstrate antigen binding activity and in fact groups of proteins have been characterized which bind the same antigen. We have been studying two of these groups of proteins in an attempt to 1) determine the structure of the antibody combining site, and the interaction between hapten and antibody at the molecular level and 2) ascertain the type of diversity that may occur among a group of proteins which bind the same well defined hapten.

1. We have previously reported the crystallization of the Fab fragment from McPC603, a phosphorylcholine binding myeloma protein. By use of X-ray diffraction data a map of this fragment was constructed at 4.5 Å resolution which revealed many general features of the antibody molecule. The molecule was divided into two large globular domains representing the variable and constant portions of the immunoglobulin. Each domain in turn was roughly divided in half with each half representing either the light or heavy polypeptide chain. The phoshorylcholine hapten was found to bind in a cleft located between the two halves of the variable domain.

We have recently completed the determination of the entire variable region amino acid sequence of the heavy chain from McPC603. Approximately 85% of the light chain sequence has also been determined and studies are currently in progress to complete the remaining portion of the light chain. By the use of this sequence data and X-ray diffraction data at 3.1 A resolution a molecular model of this protein has been constructed. A cleft is formed at the tip of the molecule by the three hypervariable regions of the heavy chain and two of the hypervariable regions of the light chain. The hapten, phosphorylcholine, has been found to bind in this cleft with the predominant specific interactions being between the phosphate moiety of the hapten and residues in the first andsecond hypervariable regions of the heavy chain. These studies are continuing to complete the description of the antibody antigen interaction.

We have also crystallized the Fab from a galactan binding protein, J539, and similar studies have been initiated. The results of these studies will permit the comparison at the molecular level of the combining sites and general structure of two antibodies with quite different antigen binding specificity.

2. Comparative amino acid sequencing studies on four phosphorylcholine binding proteins are continuing. We have previously reported the amino terminal sequences of these proteins. The McPC603 heavy chain is the first complete variable region sequence to be determined for these proteins. Similar studies have been initiated on the heavy chains of the other anti-phosphorylcholine proteins so that comparisons as to the degree of variability among these proteins can be made. Sequence studies on three of the light chains are nearing completion and will permit a similar comparison. We have recently begun characterization of a phosphorylcholine binding myeloma protein induced in strain C57/Bl. This protein shows remarkable similarity to two of the BALB/c anti-phosphorylcholine proteins although there are clearly differences. A complete analysis of the C57/Bl protein may permit an insight into the divergence of a specific pair of germ line genes within a species.

Structural studies on a group of proteins with anti-B-(1→6) -D-galactan activity has resulted in the development of a fluorescent enhancement technique for measuring antibody affinities. By using a series of galactan derivatives the combining sites of two of these proteins have been "mapped".

As a result of structural studies of the nature of those described above experiments are being initiated for the in vitro synthesis of McPC603. If successful this will be the first demonstration of the in vitro synthesis of an antibody molecule. It is felt that continued experimentation of this nature may eventually lead to the in vitro design and synthesis of antibody molecules with pre-determined specificity.

Relationship of research to National Cancer Plan:

Approach - 1

Objective - 6

Publications:

Rudikoff, S., Potter, M., Segal, D. M., Padlan, E. A. and Davies, D. R.: Crystals of phosphorylcholine-binding fab-fragments from mouse myeloma proteins: Preparation and X-ray analysis. Proc. Nat. Acad. Sci., 69: 3689, 1972.

Sigel, M. M., Voss, E. W. and Rudikoff, S.: Binding properties of shark immunoglobulins. Comp. Biochem. Physiol. 42A: 249, 1972.

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Jolley, M. E., Rudikoff, S., Potter, M. and Glaudemans, C.P.J.: Spectral changes on binding of oliogosaccharides to murine IgA myeloma proteins. Biochemistry 12: 3039, 1973.

Padlan, E. A., Segal, D. M., Spande, T. F., Davies, D. R., Rudikoff, S. and Potter, M.: Structure at 4.5A resolution of a phosphorylcholine-binding fab. Nature New Biol. 245: 165, 1973.

Rudikoff, S., Mushinski, E. B., Potter, M., Glaudemans, C. P.J., and Jolley, M. E.: Six BALB/c IgA myeloma proteins that bind β-(1→6)-D-galactan: Partial amino acid sequences and idiotypes. J. Exp. Med. 138: 1095, 1973.

Edelhoch, H., Pollet, R., Rudikoff, S. and Potter, M.: Changes in optical parameters of myeloma proteins with phosphorylcholine binding. J. Biological Chem. In press.

Barstad, P., Rudikoff, S., Potter, M., Cohn, M., Konigsberg, W., and L. Hood: Immunoglobulin structure: Amino terminal sequences of mouse myeloma proteins with phosphorylcholine binding activity. Science. 183: 962, 1974.

Lieberman, R., Potter, M., Mushinski, E. B., Humphrey, W., and S. Rudikoff. Genetics of a new IgV_H (T15 Idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. J. Exptl. Med. In press.

Padlan, E. A., Segal, D. M., Cohen, G. H., Davies, D. R., Rudikoff, S., and Potter, M. in Cox, C. F. (ed.): The Immune system: Genes, Receptorss, signals-proceedings of the 1974 ICN-UCLA symposium on Molecular Biology. In press.

Serial No. NCI-5554
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: A. Characterization and measurement of soluble antigens associated with human neoplasms.

B. Study of plasma cell neoplasms in mice and characterization of their immunoglobulin products.

Previous Serial Number: Same

Principal Investigator: K. Robert McIntire, M.D.

Other Investigators:
Dr. William Adams
Dr. Thomas A. Waldmann (NCI-4002)
Dr. Richard Adamson (NCI-3509)
Dr. Philippe P. Sizaret, I.A.R.C., Lyon, France
Dr. Charles G. Moertel, The Mayo Clinic, Rochester, Minnesota
Dr. William L. V. Go, The Mayo Clinic, Rochester, Minnesota
Dr. Charles L. Vogel, Emory Univ. Med. Center, Atlanta, Georgia
Dr. Elliott Perlin, NNMC, Bethesda, Md.

Cooperating Units:
I.A.R.C., Lyon, France
The Mayo Clinic, Rochester, Minn.
Uganda Cancer Inst., Kampala, Uganda
National Naval Medical

Man Years:
Total - 3.00
Professional - 2.00
Other - 1.00

Project Description:

A) Characterization and measurement of soluble antigens associated with human neoplasms, Objective 6 - Approach 1.

1. Studies of Alpha-Fetoprotein (AFP)

a. Quantitation of AFP in human disease: Further studies of serum AFP levels in patients with hepatocellular carcinoma during therapy have demonstrated that only 38% of 23 patients receiving systemic chemotherapy showed a decrease in AFP level while 83% of 6 patients receiving dichloromethotrexate by hepatic artery catheter and all 7 patients treated with surgery had a drop in serum AFP. Patients who were not treated had stable or increasing AFP levels. Further work is needed to show that the chemotherapy-induced reduction of AFP is due to tumor cell kill rather than interference with AFP production in neoplastic cells.

The continued survey of patients with gastrointestinal neoplasms at the Mayo Clinic for serum AFP elevations showed only 2 of 154 patients with nonmalignant disease to be elevated while 15% of 95 patients with gastric carcinoma, 3% of 191 with colo-rectal, 24% with pancreatic and 25% with biliary tract cancer were elevated. Patients with colo-rectal and gastric cancer were seen to have decreasing AFP levels during therapy-induced remission and rising levels during times of tumor proliferation.

The combined use of two serum tumor markers has been studied in patients with testicular tumors in collaboration with Dr. T. Waldmann (NCI-4002) and Dr. E. Perlin (NNMC). A large survey (close to 200 patients) indicate that over 3/4 of all patients with testicular tumors containing germ-cell elements will have one or both of these markers elevated in circulating blood. Serial measurement of both AFP and human chorionic gonadotropin (hCG) appear to be more helpful than measurement of either one alone. The cell of origin of these two proteins may be different and selective proliferation of one cell line, particularly after therapy, may be reflected by the emergence of either or both tumor markers. Using both to monitor treatment may avoid false assumptions arising from an apparent discordance of tumor growth and serum concentration of either marker.

The combination of two tumor markers, AFP and carinoembryonic antigen (CEA), have been simultaneously measured in serum of 100 colorectal and 45 gastric cancer patients. 71% of colorectal cancer patients had elevated CEA, only 3 had elevated AFP, but 2 of these 3 also had elevated CEA. Combined assays in colorectal cancer therefore, were only slightly better than CEA alone. In 45 patients with gastric cancer 10 had elevated CEA (22%) and AFP was elevated in 12 (27%) and only one patient had elevations of both. The combined assays, therefore, meant 21 of 45 (47%) of patients had a potential marker for following therapy. In both forms of cancer the elevation of AFP appeared independent of the presence or absence of liver metastasis.

b. Carcinogen-induced hepatoma in primates: The serum AFP levels in biopsy-proved hepatoma were scattered over a wide range, but were elevated in all monkeys induced by N-nitrosodiethylamine. Efforts to reduce the AFP by chemotherapy of the hepatoma have not been universally successful, but surgical removal of the majority of tumor has produced an immediate and dramatic decrease in AFP.

c. Metabolism of AFP: Native and purified AFP both have a T1/2 of 3.5 - 4 days when injected into normal adult monkeys and agrees well with AFP decay in newborn monkeys. Monkeys with hepatoma and elevated serum AFP demonstrate a somewhat faster T1/2. *In vitro* synthesis of AFP has been measured in fresh biopsy specimens maintained in organ culture using the incorporation of ^{14}C-labelled amino acids into specifically (immuno-) precipitable AFP. Linear synthetic rates for 24-72 hours may be indicative of in vivo synthetic rates.

d. Immunochemical studies of AFP: Work has begun which indicates clearly there are at least two electrophoretic variants of monkey AFP and probably also of human AFP. Work in progress is aimed at defining the reason for these variants.

2. Studies of Lung Tumor Associated Antigens: Objective 5 - Approach 4

Sixteen rabbits have been immunized with saline extracts of human lung tumors. After complete absorption with normal serum and extracts of normal lungs, there were 3 antisera with precipitin activity against lung tumor extracts, apparently reacting with the same antigen. In collaboration with Dr. P, Sizaret (IRAC) we have tested extracts of a large number of lung tumors and 59/74 had sufficient antigen present to give a precipitin reaction. Extracts of all normal tissues, including lung, were negative, except spleen. The majority of other tumor extracts were negative but some were positive and included melanoma, Burkitt's lymphoma, reticulum cell sarcoma, osteogenic sarcoma and rhabdomyosarcoma. The antigen was not detected in fetal lungs, fetal serum, normal adult serum or the serum of patients with lung tumors. The antigen did not seem to be correlated with tumor histology or blood group of the patient.

Current work is aimed at isolation of the antigen by affinity column chromatography so that a sensitive and quantitative assay for the antigen may be developed using the techniques of radioimmunoassay.

B. Mouse immunoglobulins

1. Immunoglobulin (Ig) markers on plasma cell surface: Objective 3 - Approach 5.

Previous studies have demonstrated the presence of Ig of 2 different classes on the surface of cells of murine oil-induced plasma cell tumors which secrete protein of the same Ig class. Virtually all of the tumors also exhibit kappa type Ig light chain on the cell surface. Two of these tumor lines which have been adapted to culture by Dr. G. David Trisler were examined and found to retain the expression of membrane-associated kappa chain in long term cell culture.

In related studies still in progress, we have found evidence for cytophilic host antibody on cells of nearly half of these tumors, and on cells representing all but one of the Ig classes. The addition of complement alone is sufficient to lyse most of these cells. We are currently attempting to define the suspected host antibody and its relationship with the tumor cell.

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Waldmann, T. A., McIntire, K. R., Suer, M. E., Blackman, M. A. and Princler, G. L.: Radioimmunoassay of α-fetoprotein in the diagnosis of malignancy. Miles International Symposium, VII, 1974. In press.

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Serial No. NCI-5557
1. Laboratory of Cell Biology
2. Cellular and Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immune Response to Tumor Specific and Other Cell Surface Antigens

Previous Serial Number: Same

Principal Investigator: Ronald B. Herberman, M.D.

Other Investigators: Chou-Chik Ting, M.D., Robert K. Oldham, M.D., Devron Char, M.D., Moshe Glaser, Ph.D., Holger Kirchner, M.D., Guy Bonnard, M.D.

Cooperating Units: Hematology and Supportive Care Branch, NCI; Viral Leukemia and Lymphoma Branch, NCI; Surgery Branch, NCI; George Washington University, Contracts NIH 69-2176 and 72-3251; Georgetown University School of Medicine; Litton Bionetics, Inc., Contracts NIH 69-2160, 72-3227 and 72-3878; National Naval Medical Center

Man Years:

Total:	12-1/2
Professional:	7-1/2
Other:	5

Project Description:

Objectives: The objectives of this project are: (1) to detect tumor specific and tumor associated antigens and quantitative changes in normal antigens on malignant cells; (2) to detect antibodies and cellular immune reactions to tumor antigens; (3) to evaluate the diagnostic, prognostic and therapeutic significance of humoral and cellular immune response to tumor antigens; (4) to develop and evaluate immunodiagnostic assays for human cancer; (5) to measure general immunological competence of cancer patients; (6) to detect delayed hypersensitivity reactions and other immunological reactions to human tumor antigens; and (7) to isolate and characterize tumor antigens and other cell surface antigens.

Methods Employed: Sera and cells are obtained from patients and control subjects. A variety of normal and tumor cell lines are maintained in tissue culture. Inbred strains of mice and rats, and transplantable tumors induced by viral, chemical or unknown agents are used in model systems. Antigen-antibody reactions are measured by complement-dependent cytotoxicity reactions and by an indirect ^{125}I-labeled antibody technique. Cellular immune reactions are measured by skin tests, mixed lymphocyte cultures, migration inhibition, and lymphocyte-mediated cytotoxicity assay. Human and animal tumor and normal cell membrane and soluble fractions are prepared for antigenic and biochemical studies. Tumor cells and normal lymphocytes are tested for membrane receptors by immunofluorescence and by rosette formation with sheep erythrocytes.

Major Findings:

I. Tumor Immunity in Animal Models:

a. Almost all laboratories performing immunological experiments in this system have used MSV stocks which caused transient tumor growth and then regression in adult mice. It has been possible to identify stocks of MSV which cause true progressive growth of tumors and death of the animals in most adult mice. This provides an important system to contrast with the usual regressing system. In addition it has been possible to set up an MSV system in nude mice, where tumor growth may occur without the influence of a T cell response.

b. The kinetics of the cell-mediated response to MSV have been carefully worked out. Most previous studies have involved measurements at only one or two time points. In the ^{51}Cr release assay, it has been found that draining lymph nodes become strongly positive within eight days after virus inoculation. Peak activity is seen in the spleen at 14 days and then rapidly declines. A similar pattern is seen with non-draining lymph nodes. In mice bearing true progressor tumors, a similar, but lower, response has been seen. In no case has appreciable reactivity been observed in this assay after 25 days. This pattern is in contrast to findings in the microcytotoxicity assay, where persistent reactivity has been observed. In our previous study of the cell-mediated immune response to a Gross virus-induced leukemia in rats, it appeared that the major difference between the two assays was the time of incubation in vitro. To examine this possibility in the MSV system, a more sensitive and quantitative long term assay of cytotoxicity, release of ^{125}IUDR, was performed. The kinetics in this assay were almost identical to those seen with the short term ^{51}Cr release assay.

A major problem in performing careful studies of kinetics of cell-mediated immunity, not only in this system but in other systems, has been the considerable day-to-day variations among animals in the level of cytotoxicity. To better standardize the ^{51}Cr release assay and to allow better comparisons between experiments, efforts were directed toward freezing all components of the system. It has been possible to successfully preserve the immune lymphocytes and the target cells. Using the frozen materials, the day-to-day variation has been reduced to approximately 5 percent.

Cell-mediated immunity has also been studied in this system by the lymphocyte stimulation assay. It has been found that during the period of transient tumor growth, the ability of B cells to respond, even to mitogens, is markedly depressed. When the tumor regresses, reactivity returns to normal. With progressive growing tumors the defect remains marked until the death of the animals. This has been shown to be clearly due to the presence of a suppressor cell in the spleen, which appears to be an activated macrophage. Suppressor activity can be largely removed by treatment of the cells with carbonyl iron and removal of phagocytic cells by a magnet, or by passage of the cells over a rayon column. Before such treatment the spleen cells are poorly stimulated by mitomycin treated Rauscher or Moloney leukemia cells. After this treatment, better activity is seen.

c. The kinetics of appearance of humoral antibodies have also been studied. In the ^{51}Cr release assay, antibody titers gradually rise after 20 days to a peak after 30 days in regressor animals. The kinetics have not been yet carefully examined in progressor animals. By the isotopic antiglobulin technique (IAT), significant response can be detected within five days after immunization and the peak occurs at 14 days. Extensive studies have also been performed looking for serum factors which could inhibit the cell-mediated ^{51}Cr release assay. To date only small amounts of inhibition have been observed, even with high titered antisera or with sera from mice bearing large progressor tumors.

d. It has been found that C57Bl/6 mice, in response to inoculation with MSV, react strongly in the cell-mediated ^{51}Cr release assay. In contrast BALB/c mice, the strain most widely used for microcytotoxicity studies with MSV, react either poorly or not at all, as measured by the ^{51}Cr release assay. BALB/c x C57BL/6 hybrid mice have shown intermediate results.

e. By a competitive inhibition assay, using unlabelled target cells, the major antigen detected in the cell-mediated ^{51}Cr release assay in B6 mice, has been shown to be related to expression of an antigen related not to MSV or to FMR leukemia viruses, but rather to mouse endogenous virus. This antigen is expressed on a variety of in vivo passaged C57Bl/6 tumor cells, but has not been found on most BALB/c in vivo tumor cells. In contrast, the antigen has been found on a large number of BALB/c cell lines growing in vitro. It appears that this difference in antigenic expression in the BALB/c is due to antigenic modulation. The ability of the BALB/c mice to cause modulation of the antigen may account for the poor immune responsiveness to inoculation of MSV.

f. Attempts have been made to better understand the various components of the cell-mediated immune response to MSV. George Klein and others had suggested that the principal effector cell reacting to H2 antigens was the T cell, but that to weaker tumor associated antigens, B cells constituted the major effector cell population. However, we clearly found that all of the cell-mediated cytotoxic reactivity, as measured by the ^{51}Cr release assay, could be eliminated by treatment with anti-theta and complement. The experiments with immunosuppression have been performed to further dissect out the nature of the cells involved in the cell-mediated immune response against MSV. Such studies were particularly appropriate because very little information is currently available on the effect of various chemotherapeutic agents on cell-mediated immune responses to tumor associated antigens. Almost all of the data obtained to date with these agents, both in experimental animals and in man, have been with other antigens. These experiments have provided evidence for differential effects of various agents on either the induction of immunity or more on the effector cells. There have been some interesting differences between the effects of some agents on tumor growth as opposed to their effects on in vitro reactivity measured in the ^{51}Cr release assay.

g. Of central importance in studies of tumor immunity is the relationship of the results obtained in in vitro assays to the in vivo response to tumor growth. An adoptive transfer system has, therefore, been established to determine the effect of various cell populations, at various times after immunization, on resistance to tumor growth.

h. It has been found that some normal mice and rats have cell-mediated reactivity, measured in the ^{51}Cr release assay, against virus-induced leukemia cells. We have found that there is considerable variation in natural reactivity among animals, and particularly between strains of mice. C3H mice have had the strongest reactivity, followed by BALB/c, with C57Bl/6 having the least reactivity. The amount of natural reactivity detected varies considerably according to the target cell line. RBL-5 cells growing in tissue culture are considerably more sensitive to lysis by normal cells than are the ascites cells. However, reactivity against both the tissue culture and ascites are parallel, i.e. the spleen cells of a mouse which react most strongly against the tissue culture cells also react most strongly against the ascites. The natural reactivity is age related: No reactivity has been detected before four weeks of age; there is a peak of reactivity between six to eight weeks of age, and then a gradual decrease in activity. The natural reactivity in germ-free mice and in nude mice is approximately the same as that in conventional mice. Specificity of antigen: The detected antigen appears to be related to mouse endogenous viruses. However, the pattern of inhibition has been somewhat different than that observed with spleen cells from mice which have been immunized by MSV. Somewhat different specificities have also been detected on the RBL-5 tissue culture cells compared to RBL-5 ascites or to another leukemia target cell, RL♂1. The effector cell has been found not to be affected by treatment with anti-theta serum and complement. From this evidence and the observation of strong reactivity in nude mide, a non-T cell is thought to be responsible for the activity. However, the nature of the responsible cell has not been clearly determined: Reactivity has not been affected by treatment with carbonyl iron or by passage through a rayon column or by addition of a potent antiglobulin reagent (which has been shown to effectively inhibit lymphocyte dependent antibody killing).

i. We have measured cell-mediated immunity to the W/Fu rat Gross virus-induced lymphoma (C58NT)D by the ^{51}Cr release cytotoxicity assay and by the mixed lymphocyte tumor interaction. We have compared kinetics of immune responses measured by these assays, and also the specificity of the antigens detected in each assay. The relationship of the reactivities measured in vitro to in vivo resistance against tumor growth, by performance of adoptive transfer experiments, was determined. Direct cytotoxic reactivity, as measured by the ^{51}Cr release assay, has shown that there is a peak of cytotoxicity at ten days after tumor cell inoculation with a rapid decline to negligible levels after 15 days. In contrast, the lymphocyte stimulation assay with mitomycin treated tumor cells has not become positive before 15 days, and reaches a maximum between 25 and 40 days. With progressive tumor growth the cytotoxic response is increased relative to the regressor system; in contrast the mixed lymphocyte tumor cell interaction in progressor rats is uniformly negative. Despite these marked differences in the kinetics in each assay, both responses have been shown to be due to T cells (complete inactivation by a specific anti-T cell serum plus complement). Studies of the specificity of the antigen detected in the cytotoxicity assay have given results similar to those obtained with the mouse MSV system. The antigen appears to be related to endogenous rat C-type virus.

II. Tumor Immunity in Man:

a. A series of assays for measuring the general cell-mediated immune competence of cancer patients have been established. These assays are: enumeration of the percent of T cells in the peripheral blood by rosette formation with sheep erythrocytes, cytotoxic reactivity against human lymphoid cell lines, and lymphocyte stimulation by mitogens, antigens, and mixed lymphocyte cultures. A major problem with these assays for monitoring has been the day-to-day technical variations in the procedures. It has been possible to cryopreserve human lymphocytes and retain their functional activities in each of these assays. This has resulted in a considerable decrease in the variability of the assays and allows a consistent standard to be included in all of the assays.

b. Further studies have been performed with the direct leukocyte migration inhibition assay. The leukocytes of patients with carcinoma of the breast and with malignant melanoma have been shown to be specifically inhibited by hypertonic KCl extracts of autologous or allogeneic tumors of the same histological type. Reactivity has been found in patients with localized disease and also in patients with widespread, metastatic disease.

c. Lymphocyte stimulation assays have also been performed with these KCl extracts of tumors. It was found that it was not possible to use allogeneic extracts for measurement of tumor immunity, since these extracts also produced appreciable stimulation of many control individuals.

d. Cytotoxic reactivity of the lymphocytes of patients with various carcinomas was evaluated by microcytotoxicity assays and by cytotoxicity assays using ^{125}IUDR and 14C-proline. Thus far these assays have not been practical for measurement of specific immunity to tumor associated antigens. Because of the reactivity of many normal individuals against tissue culture cell lines of human tumors, in many cases some normal controls showed more reactivity than the cancer patients run in the same test.

e. Patients with neoplastic disease have been shown to have delayed hypersensitivity reactions to extracts of tumor cells. Reactions to membrane extracts of tumor cells are being used to follow the course of disease of patients and correlations with clinical parameters are being made. The patients have been tested simultaneously by skin reactivity and by leukocyte migration inhibition assays. Thus far, these assays have shown good correlation; however, the skin tests have appeared to be more sensitive.

f. Soluble skin reactive antigens have been isolated from the membranes of breast tumors and of malignant melanoma. In each of these diseases, two separate skin reactive antigens have been isolated by polyacrylamide gel electrophoresis. One antigen has given reactions only in patients with the specific disease; the other antigen has shown wider reactivity, in patients with the particular disease and also in patients with other types of cancer. It seems likely that the latter antigen is a tissue associated antigen rather than tumor associated.

Significance to Biomedical Research and the Program of the Institute:

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. The sensitive assays for humoral and cell-mediated immunity which have been developed in the laboratory have already proved useful in studying the immune response to animal and human tumors.

The ability of our laboratory to perform multiple assays of humoral and cell-mediated immunity to tumor antigens should allow detailed dissection of the role of the immune response in limiting or preventing tumor growth. These assays may also be useful in immunodiagnosis of cancer and in measuring changes in immunity, in response to chemotherapy or immunotherapy.

The available immunological techniques have also allowed progress in the solubilization and isolation of tumor associated cell surface antigens. Such studies will permit a comparison of the physicochemical nature of tumor antigens and normal histocompatibility antigens and may provide material for active immunization of animals and tumor patients against their tumors.

Proposed Course of Project:

Studies are in progress to further understand the immune responses of experimental animals and of patients to tumor associated antigens.

In experimental animals, further studies will be performed on the nature of the cells involved in various immune fractions and on their mechanism of action. Studies on the interrelationships between the different assays will be continued.

Of particular emphasis will be the search for possible differences in the specificity of the antigens detected in each of the assays, and on the role of each of the in vitro reactivities for in vivo protection against growth of tumors. The latter question will be examined by the extensive use of adoptive transfer experiments.

Studies of cell-mediated immunity in cancer patients will be continued. Assays of general immunological competence and of reactivity to tumor associated antigens will be used to monitor the response of patients to immunotherapy with BCG or with tumor cells. Studies on the inter-relationships between the various assays of reactivity to tumor antigens will be continued. Attempts will be made to better understand the specificity of the antigens detected in each of these assays. The results of each assay will be correlated with clinical parameters, to determine which assays are most useful for prognosis.

Honors and Awards:

Ronald B. Herberman
1973, Annual Award in Biological Sciences of the Washington Academy of Sciences

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Serial No. NCI-5558-1
1. Laboratory of Cell Biology
2. Cellular & Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immune Response to Tumor Associated and Other Cell Surface Antigens

Previous Serial Number: Same

Principal Investigator: Chou-Chik Ting, M.D.

Cooperating Units: Cell Physiology and Oncogenesis Section, Laboratory of Biochemistry, NCI; Litton Bionetics, Inc., Contract NO1-CB-23878

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: The objectives of this project are: (1) early detection of antitumor antibody in murine tumor system; (2) in vitro cell-mediated immunity (CMI) to Friend leukemia; (3) correlation of various antigens defined by in vitro assays to tumor associated transplantation antigen; (4) expression of fetal antigens in fetal tissue culture cells.

Methods Employed: Tumor cells transformed by oncogenic viruses, chemicals, or unknown agents are kept in tissue culture or as transplanted tumors in mice or rats. The isotopic antiglobulin technique (IAT) was used to perform serological studies. The ^{125}IUDR lymphocyte cytotoxicity test was used to study in vitro CMI, and tumor transplantation experiments were used to study in vivo CMI.

Major Findings:

I. Early Detection of Antitumor Antibodies:

These experiments were designed to set up a model for immunodiagnosis of cancer.

a. SV40 tumors: CBF_1 mice were implanted at foot pad with Trocar pieces of syngeneic SV40 tumor SVT_2. The tumors became grossly recognizable at two weeks after implantation; they were removed after three weeks in half of the mice, and tumors were left to grow in the other half. Sera were obtained from these mice at different intervals after tumor implantation and surgical excision. Testing by IAT, it was found that anti-SV40 tumor antibody could be detected at two weeks after tumor implantation, which was the time that tumors became grossly recognizable. The level of antibodies rose with tumor progression and declined after amputation but remained detectable up to 11 weeks after implantation, when the experiment was terminated. Testing by ^{51}Cr cytotoxicity test (CT), antitumor antibody could be detected at four weeks after tumor implantation; the level of antibodies also rose with tumor progression but became undetectable at two weeks after amputation. In comparison of the sensitivity of the two serological techniques, IAT was found to be 100-400 times more sensitive than CT.

b. Friend leukemias: C57BL/6 mice were inoculated intraperitoneally with 1×10^2 FBL-3 cells, a syngeneic Friend leukemia, and DBA/2 mice were inoculated subcutaneously with 1×10^2 FLC cells, a syngeneic Friend leukemia. Antitumor antibodies to these tumor cells could be detected one-two weeks prior to tumor development or at the time of tumor development. The antibody levels rose with tumor progression.

II. In Vitro Cell-Mediated Immunity to Friend Leukemia as Measured by ^{125}IUDR Lymphocyte Cytotoxicity Test:

Previous serological studies performed with IAT revealed that three antigenic specificities could be defined by the sera produced in C57BL/6 mice immunized with syngeneic Friend leukemia FBL-3. These specificities are: type specific (Friend) antigen, FMR common antigen, and a broadly cross reactive antigen of fetal nature. In vivo tumor transplantation experiments revealed that only the first two antigens were correlated with tumor associated transplantation antigen (TATA).

a. Correlation of the antigenic specificities defined by ^{125}IUDR assay, to those defined by serological studies and tumor transplantation experiments: Three antigenic specificities could be defined by ^{125}IUDR assay: (1) Lymphocytes from FBL-3 immune mice always gave higher degree (3 fold more) of cytotoxicity against FBL-3 than against leukemias induced by Rauscher virus (RBL-5) or Moloney virus (MBL-2) which suggested that there was a type specific (Friend) antigen in addition to FMR common antigen. (2) Occasionally, low grade cytotoxicity could be obtained against a chemical induced leukosis EL.4, which indicated a non-FMR cross reactive antigen. Therefore, these specificities correlated with the serological studies. The detection of cross reactive antigen by ^{125}IUDR assay indicated that either this assay was more sensitive than in vivo tumor transplantation experiments, or it was not correlated with TATA.

b. Specificity: The specificity of the reaction was revealed by the difference in reactivity of the FBL-3 immune lymphocytes against various leukemic cells as shown above. Furthermore, lymphocytes from EL.4 immune mice were generally not cytotoxic against FMR leukemic cells, but would give low grade of cytotoxicity against EL.4 cells. The difference in the reactivity of these cells could not be simply attributed to their susceptibility to lymphocyte cytotoxicity; lymphocytes obtained from mice immunized with Moloney sarcoma virus gave the same degree of cytotoxicity against FMR leukemias and EL.4 cells.

c. Kinetics of immune response: Significant cytotoxicity could be detected at five days after immunization, peaked at 10-14 days, then gradually declined but remained reactive up to 32 days or more.

d. Correlation of the lymphocyte cytotoxicity to status of tumor growth: Significant cytotoxicity to FBL-3 cells could only be obtained with lymphocytes from mice immunized with 5000 R x-irradiated FBL-3 cells, or immunized with viable FBL-3 cells by subcutaneous injection which only resulted in temporal tumor growth. No significant cytotoxicity was obtained with lymphocytes from tumor bearing mice. Therefore, the lymphocyte cytotoxicity measured by ^{125}IUDR assay seems to be correlated with the status of tumor growth.

III. Expression of Fetal Antigens in Fetal Tissue Culture Cells (in collaboration with Dr. K. K. Sanford):

These experiments were designed to study the expression of fetal antigens in fetal tissue culture cells, its relationship to spontaneous transformation, and the possible usefulness of these cells as the standard source of fetal antigens.

a. Fetal antigens were retained in fetal tissue culture cells for up to five years of long term culturing. The expression of fetal antigens varied with cell lines, and also varied with different strains of mice.

b. There was no correlation between the expression of fetal antigen and spontaneous neoplastic transformation.

c. Since fetal antigens are not found in normal adult tissue, and some protection against a MCA-induced sarcoma in B6 mice could be achieved by immunization with fetal antigens, these tissue culture cells may be useful as the source of fetal antigen for immunodiagnosis or immunotherapy.

Significance to Biomedical Research and the Program of the Institute:

There are numerous in vitro assays available to detect the immune response of tumor associated antigens and other antigens. The tumor associated transplantation antigen (TATA) is of prime importance in the induction of in vivo tumor immunity. Our studies indicated that only some of the tumor associated antigens defined by in vitro assays were correlated with TATA. Therefore, this correlation should be carefully evaluated before immunoprophylaxis or immunotherapy are attempted.

IAT has proved to be very sensitive in detection of antitumor antibody prior or at early stage of tumor development. The levels of antibody correlated very well with the status of tumor growth. These findings indicated that sensitive serological techniques can be used for immunodiagnosis of cancer, and for monitoring the status of tumor growth.

Fetal antigens were found to be retained in long term fetal tissue culture cells. These cells may be useful as the standard source of fetal antigens for immunodiagnosis or immunotherapy.

Proposed Course of Project:

I. Early Detection of Antitumor Antibody in Mice with Primary, Autochthonous Tumors:

We have been able to detect antitumor antibody prior or at the early stage of growth of transplantable tumors (SV40 tumors, Friend leukemia). In order to set up a closer model for immunodiagnosis of human cancer, we plan to use primary, autochthonous tumors in mice. We shall use the tumors induced by Moloney sarcoma virus in C57BL/6 mice and the spontaneous mammary tumors in C3H/HeN mice.

II. CMI to Friend Leukemia:

We shall further study the specificity of the in vitro CMI to Friend leukemia, as measured by ^{125}IUDR assay, with adsorption experiments. In vivo adoptive transfer experiments will also be performed to study the correlation of in vitro

CMI to in vivo tumor immunity. The interaction of antibody to in vitro CMI will also be studied.

III. Mouse Mammary Tumors:

Due to our previous difficulty to perform in vivo tumor transplantation experiments with C3H mouse mammary tumors (MMT), we shall concentrate on serological studies of these tumors. C3H and C3Hf mice will be immunized with C3H MMT. Sera will be obtained from hyperimmunized mice and from tumor bearing mice. IAT will be used to perform the serological studies, to determine whether a common tumor associated cell surface antigen unrelated to MTV virion can be detected in various MMT. (We can use some MTV(-) MMT as test cells.)

Publications:

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2. Ting, C.C., Ortaldo, J.R. and Herberman, R.B.: Serological analysis of the antigenic specificities of SV40 transformed cells and their relationship to tumor associated transplantation antigen. J. Nat. Cancer Inst., in press.

3. Ortaldo, J.R., Ting, C.C. and Herberman, R.B.: Modulation of fetal antigen(s) in mouse leukemia cells. Cancer Res., in press.

4. Ting, C.C. and Herberman, R.B.: Immune response to Friend virus-induced leukemia in C57BL/6 mice. I. Serological analysis of the antigenic specificities. Submitted for publication.

5. Ting, C.C., Shiu, G., Rodrigues, D., and Herberman, R.B.: Immune response to Friend virus-induced leukemia in C57BL/6 mice. II. Cell mediated immunity in vivo and in vitro. Cancer Res., in press.

Serial No. NCI-5596
1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pathogenesis of plasma cell neoplasia in mice; immunochemical characterization of antigen-binding myeloma proteins; genetic basis of immunoglobulin diversity

Previous Serial Number: Same

Principal Investigator: Michael Potter, M.D.

Other Investigators:
Dr. R. Asofsky (I:LGAR-116c)
Dr. R. Lieberman (I:LCI-15c)
Dr. S. Rudikoff (NCI-5553)
Dr. David R. Davies (A:LMB-7)
Dr. C.P.J. Glaudemans (A:LC-7)
Dr. Don W. Bailey (Roscoe B. Jackson Lab., Bar Harbor, Maine)
Dr. Marshall Sklar (I:LVD)

Cooperating Units: None

Man Years:
Total: 4
Professional: 1
Other: 3

Project Description:

I. Pathogenesis of plasma cell tumor development.

Plasma cell tumors can be induced in 60 to 70% of BALB/c mice by the intraperitoneal injection of various mineral oils or pure hydrocarbons (e.g. pristane). With this model system 3 important general factors appear to participate in plasmacytoma development: 1) Genetic susceptibility, revealed by the strain susceptibility of inbred strains BALB/c and NZB and the resistance in most other strains (C57BL, DBA/2, C3H, ALN); 2) An abnormal tissue microenvironment - the mineral oil granuloma which promotes plasmacytoma development; 3) An initiating factor which could be due to replication of an intrinsic latent virus or a spreading somatic infection by an endogenous virus; 4) Antigenic stimulation.

A. Genetic factors in mineral oil induced plasmacytoma formation.

1. Search for susceptibility genes.

Strain C57BL/6 mice are resistant to plasmacytoma formation by mineral oil. The availability of the 7 Bailey Recombinant strains derived from a cross between C57BL/6 and BALB/c provides a potential means to identify genes involved in susceptibility. The 7 Bailey strains have been tested initially for susceptibility to BAYOL-F and the CXBG and CXBJ strains developed a high incidence of tumors. The CXBD strain was inconclusive, the others were essentially negative. The study was complicated by a high incidence of non-specific deaths, and is now being repeated using pristane which induces tumors in a shorter time. Using pristane the CXBG, CXBJ, CXBE, and CXBD strains have been found to be susceptible, CXBH, CXBI and CXBK resistant. This result suggests a contribution of H-7. D. W. Bailey has a congenic stock with the BALB/c H-7 gene on the resistant C57BL. These mice are being bred at NIH and tested for susceptibility.

The Bailey RI strains have been tested also by the new rapid induction method using one injection of pristane and Abelson virus 40 days later. By this method CXBG, CXBJ, and CXBD are susceptible, the others are resistant. CXBE is resistant to virus infection.

Using the rapid method it has been found that DBA/2 and (BALB/c x DBA/2)F_1 are virus susceptible, plasmacytoma resistant. This cross will permit analysis of F_2 mice for susceptibility - a project now in progress.

B. Abnormal tissue microenvironment

Continuing studies on the role of the oil granuloma continue. A rapid assay system using a primary tumor stored in the frozen state is being developed. With an assay system attempts will be made to isolate the growth stimulating factor. The effect of cortisone on oil granuloma stimulation is being examined.

Dr. Maya Ran, a Fogarty International Fellow, is studying the mechanism of local immune suppression in the peritoneal oil granuloma. Macrophages actively remove antigens from the surface of cells rendering the cells, insensitive to cytotoxicity by antibody and complement.

The role of the oil granuloma in rapid plasmacytoma development by Abelson virus is being studied. Optimal susceptibility for plasmacytoma development requires the presence of the oil for 30 to 40 days. The underlying changes in the cell population during this period are being examined.

C. Antigenic Stimulation

Pathogen-free BALB/c mice, like germfree BALB/c mice are resistant to plasmacytoma development by pristane. We are searching for a means for restoring susceptibility. The effect of endotoxin on restoring susceptibility of PF mice will be tested in the rapid induction method. PF-BALB/c mice are generally resistant to plasmacytoma induction by Abelson virus and pristane. Pathogen-free BALB/c mice will be given multiple injections of endotoxin.

This agent is known to be a nonspecific mitogen for B-cells and may amplify the precursor population.

D. Test of pure pristane

Pristane contains small amounts of olefin contaminants, a pure pristane sample provided by Dr. Max Blumer will be tested for activity and compared with commercially obtained pristane.

II. Immunochemistry of myeloma proteins. Genetic basis of diversity. Structure of combining site of antigen binding myeloma proteins

A. Structure

Groups of BALB/c myeloma proteins that bind phosphorylcholine. β1-6D galactan, α 1-3, α 1-6 dextran, β 2→6 fructosan, DNP have been collected and studied both structurally and antigenically.

Structural studies in collaboration with Dr. Stuart Rudikoff and Dr. David Davies' group involve determination of primary structures, and 3-dimensional structures by X-ray crystallography. A 3.1 Å electron density map of the phosphorylcholine binding myeloma protein McPC 603, based on the complete sequence of the V_H and 80% of the V_L has been completed. The crystal was made with the ligand in site and this permits the definition of the binding site. A second crystal is being prepared with J 539. This information provides the means for a new approach to immunochemistry.

Essentially, the Ig combining site is a localized at one pole of the Fv, in a continuous surface bounded by the hypervariable segments from the V_L and V_H. Since the internal architecture of V_L and V_H domains in both mouse and man is extremely similar, by knowing the 3-dimensional structure of the binding site it will become possible to modify the structure of antibodies.

Much more must be learned about natural antibodies however and mouse system provides the most optimal approach available. Collaborative studies with C.P.J. Glaudemans have demonstrated the J539 binding site can be mapped in detail. A model of this antibody will prove invaluable in determining the structural basis of specificity.

B. Genetics

The genetics of IgV_H genes in the mouse has developed extensively in the last year chiefly through the availability of congenic strains (CB-20, AL-20) of mice developed in this laboratory. A new genetic marker, the T-15 idiotype, has been found in normal immunoglobulins (in collaboration with Rose Lieberman). The gene controlling the T15 idiotype has been linked to the IgC_H locus and structural studies indicate the gene is in fact an IgV_H gene. This was facilitated by finding a CB-20 directed phosphorylcholine binding myeloma protein. We are continuing to search for new genetic markers and expect to begin mapping the IgV region in the mouse in the next year.

Publications:

Rudikoff, S., Mushinski, E. B., Potter, M., Glaudemans, C.P.J. and Jolley, M.E.: Six BALB/c IgA Myeloma Proteins that Bind β-(1 → 6)-D-Galactan. J. Exp. Medicine 138: 1095-1106, 1973.

Jolley, M. E., Rudikoff, S., Potter, M. and Glaudemans, C.P.J.: Spectral changes on binding of oligosaccharides to murine immunoglobulin A myeloma proteins. Biochemistry 12: 3039-3044, 1973.

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Padlan, E. A., Segal, D. M., Spande, T. F., Davies, D. R., Rudikoff, S. and Potter, M.: Structure at 4.5Å resolution of a phosphorylcholine-binding Fab. Nature New Biology 245: 165-167, 1973.

Lieberman, R., Potter, M., Mushinski, E. B., Humphrey, J. W. and Rudikoff, S.: Genetics of a new IgV_H (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. J. Exp. Med., 139: 983-1001, 1974.

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

April 1, 1973 - March 31, 1974

The Surgery Branch has continued the dual responsibility of providing surgical consultative and operative assistance to the multidisciplined intramural clinical units of the National Institutes of Health while developing its own laboratory and clinical research programs. Approximately 60% of the Surgery Branch staff's activities are consumed by clinical responsibilities. During the past 12 months, 701 individually submitted surgical consultations were received, in addition to supervising 1131 requests for consultative services from extramural consultant sources. These figures include only the original consultative requests and are not inclusive of the tremendously larger total number of visits that were repetitively made to follow up the surgical consultative recommendations or surgery itself. Surgical procedures in the operating room were performed upon 818 patients during the past year, of which 328 were performed as part of the specific clinical research program of the Surgery Branch itself.

The ongoing clinical investigative program of the Branch can be categorized into four areas of chief interest: Cancer of the soft tissue pelvic structures, head and neck area, soft tissue and bone sarcomas, and melanomas. Each of these areas of interest have incorporated into them an ever increasing therapy trend. There is expanded programming with radiotherapy, chemotherapy (cytotoxic and anticoagulative agents), cryotherapy, and a surge of collaborative monitoring through immunological surveillance. Pelvic Structures: The role of radical surgery in the successful management of patients with radiation recurrent cancer of the cervix and vagina has been well established. A 45% actuarial five-year survival has been obtained in 127 patients with recurrent treatment failure disease, and in 101 patients with locally advanced primary cancer the survival has been 65%. A most significant aspect of this program has been the refinement of techniques which allow a safe and satisfactory means of diverting the urinary stream through the means of using either small or large bowel isolated conduit. Following pelvic exenteration, rehabilitative procedures such as vaginal plastics continue to be successfully utilized in selected patients. Head and Neck: Local disease recurrence subsequent to treatment of cancers of the head and neck area continues to be an enigma. Based upon new evidence indicating that the presence of an isolated mass of tumor cells which might remain subsequent to conventional head and neck therapy might be highly sensitive to adjuvant therapy regimes, the Methotrexate study has been expanded. Preoperative use of this agent has shown an 81% primary tumor response rate without altering surgical morbidity. This is in contrast to the morbidity which has been repetitively demonstrated in adjuvant radiotherapy or radical surgery studies. Because of the morbidity potentially associated with the intracranial transfacial approach to paranasal sinus cancer, adjuvant studies have not yet been incorporated into this longstanding study program. Fifty-seven patients have now undergone the en bloc resection of their paranasal sinuses through this unique operative approach with an actuarial survival of

47% and a five-year survival of 59%. The use of extended shoulder and deltopectoral full thickness skin flaps continues under intense investigation as the modality which best allows functional and cosmetic reconstruction of the head and neck area subsequent to cancer extirpation. New innovations in total mandibular replacement have shown early success due in part to the ability to adequately protect the mandibular foreign body from predisposing to secondary infection and fistulization. The uniquely innovative study of taste and smell in patients who have undergone cancer therapy of the head and neck area continues to be carried in collaboration with the National Heart and Lung Institute. Sarcomas: Soft tissue and particularly bone sarcomas remain an enigma to the cancer therapists. The Branch experience with a limited number of each of the particular sarcomas indicates that the five-year survival following amputative surgery, with and without adjuvant therapy of several modes, is approximately 13% with osteogenic sarcomas and 60% with chondrosarcomas. The usual fibrosarcoma, rhabdomyosarcoma, and liposarcoma patient has a survival of approximately 50%. It has been demonstrated that hemipelvectomy can safely be performed either by the conventional technique or by the lateral position technique developed by the Branch, and offer satisfactory cure rates. These patients can also be functionally rehabilitated through the cooperative efforts of the physical and occupational therapist. Because of the propensity of these soft tissue and bone sarcomas to early metastases, these patients, all new admissions, are entered into an anticoagulative study whereby surgery is carried out under low dose maintenance anticoagulation. Preliminary 12-month evaluation of these study patients indicates that there may be a decreased propensity to metastases. Postoperative morbidity associated with this anticoagulative study has been shown to be directly related to the difficulty in maintaining adequately monitored levels of anticoagulation. This program has been expanded to include the addition of Adriamycin to the long-term postoperative management of those patients with high risk metastases-prone sarcomas. Melanomas: In achieving local control of the cutaneous lesions, results of standard wide-margin surgical excision are satisfactory, with a recurrence rate of less than 6%. Failure to control distant metastatic disease, however, is the primary cause of the overall mortality of 51% in five years. In order to more accurately determine the most suitable indicated for the treatment of melanoma, more accurate and comprehensive modes of staging are being developed. Subsequent to the usual clinical staging techniques, patients are receiving preliminary intra-abdominal exploration with multiple lymph node and organ body biopsies. Appraisal of the past experience of over 300 patients treated for melanoma indicates that a five-year survival of 44% is directly related to the type of melanoma that is treated and the level of invasion of the lesion, size of the primary pigmented skin abnormality, and the presence or absence of regional lymph node disease as documented by surgical resection.

The Branch has been participating and is preparing to participate in multiple efforts to evaluate the effectiveness of combinations of modalities of treatment. The Surgery Branch is currently collaborating with the Immunology Branch, and the Chemotherapy Branch in a protocol study to assess the effectiveness of immunotherapy or chemotherapy in patients with minimal residual melanoma. Patients undergo removal of the lesions and of the draining regional lymph nodes. If the regional lymph node histological examination confirms the clinical impression of involvement, the patient is then a candidate for randomization to additional chemotherapy, immunotherapy, or observation. The chemotherapy arm consists of administration of methyl CCNU. The immunotherapy arm consists of administration of BCG and allogenic cultured melanoma cells. The observation arm will provide an indication of whether chemotherapy or immunotherapy enhances survival and decreases the recurrence rate compared to surgery alone. The role of the Surgery Branch in these studies is to perform the required surgery on the portion of the patients who have definitive surgery at the NIH, to perform a portion of the immunological monitoring on all of the protocol patients, and to provide clinical followup to the patients who are not receiving chemotherapy or immunotherapy.

The possible utilization of "combination immunotherapy" is being investigated based on experiments performed in the Surgery Branch showing that in four species, including man, agents which promoted delayed hypersensitivity reaction increase the responsiveness of the circulating lymphocytes to blastogenesis by phytohemagglutinin. A combination of agents which produce a delayed hypersensitivity reaction seems to be more potent than single agents in causing the enhanced lymphocyte reactivity. It is possible that "combination immunotherapy" like "combination chemotherapy" may be more potent than "single immunotherapy" or "single chemotherapy".

The Breast Oncology Section of the Surgery Branch has joined forces with the Medical Breast Cancer Service in a Breast Tumor Section for treatment of breast cancer. Over the entire range of breast disease from early detection of suspicious breast lesions in a Breast Problem Clinic, to primary surgical therapy to be carried out by the Branch, to adjuvant chemotherapy programs, pre- and postmenopausal first recurrent chemotherapy programs, and metastatic and therapy of breast cancer patients in collaboration with the surgeons' Tumor Service. The Branch is involved also in aspiration, biopsy, primary surgical treatment, endocrine ablative procedures, and staging for all the patients entered into the service. The program is being rapidly expanded and developed along the lines of already prepared randomized protocols to be of maximum benefit to the patients in combination therapy and to achieve optimum yield of computerized data for the controlled studies jointly sponsored by the Surgery and Medicine Branches.

In collaboration with the Cytopathology Department, fine needle (22-25 guage) aspiration biopsy for the cytologic diagnosis of solid tumors is being evaluated. Findings of these studies have been again reported to the American College of Surgeons meeting and at a 1973 workshop for cytopathologists conducted in cooperation with the Cytopathology Department. Results indicate that diagnoses of cancer obtained on the basis of fine needle aspiration have been confirmed in all cases in subsequent histologic examination of excised tissue. A negative report, however, does not rule out malignancy. No untoward reactions or complications have been associated with this procedure. Use of the procedure has been extended from aspiration of subcutaneous and superficial lesions in all areas of the body to transrectal and transvaginal aspiration of deep pelvic masses and transantral aspiration of paranasal sinus tumors.

Studies carried out on patients with ileal conduits constructed for urinary diversion following urinary bladder resection for genitourinary tract carcinoma have established the normal cytology of ileal conduit urine. Abnormal ileal conduit urine cytology found in four patients has permitted detection of recurrent cancer involving the ileal conduit or upper urinary tract. Radioisotope renography studies in the evaluation of patients with ileal conduits have been shown to be a valuable aid in the followup of patients after radical pelvic surgery. Studies demonstrated the importance of the sitting position when these patients are evaluated by renography to avoid the false obstructive pattern frequently seen when the examination is carried out in the customary supine position. The renogram has been found to be a sensitive indicator of urinary tract obstruction which correlates well with excretory urography. Accordingly, long-term followup of patients following pelvic surgery can best be accomplished with periodic renograms and flat plates of the abdomen, rather than with the more complex excretory urography.

An in vitro, lymphocyte stimulation, micro-assay employing human tumor associate antigens (TAA) has been developed and used to determine the existence and the degree of cross reactivity of these antigens. The specific lymphocyte stimulation observed adds to the confirmation of the presence of TAA in human cancers Tumor antigens from malignant melanomas, sarcomas, and carcinomas of the breast and squamous carcinomas of the head and neck region were prepared by the KCL extraction method, and more recently by the cell membrane extraction method. Antigen controls were prepared from normal tissue. Significant blocking effect was noted in approximately 1/3 of the tests when autologous serum was utilized instead of pooled AB serum. Lymphocytes of black healthy controls in 2 out of 3 occasions were stimulated by the malignant melanoma antigen, which tends to confirm Hellstrom's finding in the cytotoxicity system. On several occasions lymphocytes of healthy donor origin reacted to the tumor antigen (same histological type as patient) when patient's serum was added to the culture medium, suggesting a serum factor which could render sensitivity to the normal lymphocytes.

To determine the usefulness of in vitro lymphocyte reactivity to phytohemagglutinin (PHA) and dinitrochlorobenzene (DNCB) contact sensitivity combined as determinants of the tumor status of cancer patients, cellular immune competence was determined with these assays in over 100 postoperative patients considered clinically free of tumor and compared with that of the 200 preoperative patients and normal controls. The immune reactivity of the cured cancer patients correlated with the histology of the tumor previously excised. Patients cured of squamous carcinomas had high incidences of impaired lymphocyte reactivity and DNCB contact sensitivity similar to those found in preoperative patients. Patients with sarcomas, melanomas, and adenocarcinomas, however, had lymphocyte reactivity higher than their preoperative counterparts and normal subjects, and did not display the abnormalities of DNCB contact sensitivity present in comparable preoperative groups. Among patients cured of cancer, as in preoperative patients, only those with squamous carcinomas and sarcomas had serum suppressant of lymphocyte reactivity. The persisting high incidence of impaired cellular immunity in patients cured of squamous carcinomas indicates the need for investigation of genetic and environmental factors which may be responsible for the defects and the determination of their relation to tumor induction. The intact cellular immune competence of patients clinically cured of sarcomas, melanomas, and adenocarcinomas indicates that patients treated for tumors of these histologic types who display abnormalities by the assays used may be suspect of having residual tumor.

Delayed cutaneous hypersensitivity to DNCB was determined in 268 cancer patients admitted with clinically operable tumors and compared with 143 normal subjects. Anergy or impaired reactivity to DNCB occurred in higher incidence among 80 patients who had previously received radiation therapy or who had not had completion of skin testing prior to operation than in the remaining 188 patients. Among the latter, abnormal reactivity occurred in higher incidence than normals in patients with squamous carcinoma ($p < .01$) and patients with sarcoma ($p < .05$) but did not differ statistically from normals among patients with melanomas and adenocarcinomas. Reactivity among 158 operable patients did not differ significantly from that of 30 patients found to be inoperable only after intensive evaluation ($p = .25$). Reactivity to DNCB did not correlate with the subsequent clinical course of the operable patients at a mean of 18 months (range 6 to 36 months) after treatment. Both 93% of patients who remained free of disease and 90% of patients who subsequently developed recurrences reacted to DNCB. 72% of patients with normal responses, 60% with impaired responses, and 53% who were anergic to DNCB had no evidence of recurrence during this interval (differences not significant, $p = .30$). Similarly, although patients previously irradiated had higher incidences of anergy, reactivity did not correlate with clinical course. The data indicate that DNCB reactivity determined prior to treatment in patients with clinically operable solid malignancies did not correlate with subsequent clinical course at a minimum of six months after definitive therapy.

The *in vitro* spontaneous lymphocyte rosette (T cell) assay was used to determine cellular immunologic competence in 112 patients with bronchogenic carcinoma. Among preoperative patients with localized tumors, T cell levels were significantly lower than in 237 normal controls. With advanced stages of disease, T cell levels declined progressively among patients with squamous cell carcinoma, oat cell carcinoma, and undifferentiated carcinoma, but not among patients with adenocarcinoma. Squamous carcinoma patients considered cured had persisting low T cell levels, but cured adenocarcinoma patients had normal levels. Regardless of tumor histology, 88% of preoperative patients with T cell levels below 2 standard deviations of mean control levels developed metastases within an average of 3 months after surgical resection. Serial determinations that showed a fall in T cell levels preceded the development of clinically evident metastases by an average of 2.5 months. Postoperative patients with stable or rising T cell levels have remained clinically free of disease. The data indicate that T cell levels correlate with prognosis and clinical course of patients with bronchogenic carcinoma. The assay may, therefore, provide a rational basis for the selection of patients who are at high risk for the development of metastases after surgical resection and who may benefit from the early institution of adjunctive therapy.

Peripheral blood T cell levels were determined serially in 72 patients treated with radiation therapy for bronchogenic and esophageal carcinoma. T cell levels declines progressively in all patients during therapy. The levels increased at one month after therapy in bronchogenic carcinoma patients who were tumor-free at an average of 4 months after therapy (responders). By contrast, the levels declined or failed to increase at one month after therapy in patients who developed metastases within an average of 2.5 months after therapy (nonresponders). The mean T cell levels of responders remained > 400 and increased after therapy, while the mean levels of nonresponders persisted at < 400 after therapy

($p < .0001$). 91% of patients with T cell levels < 400 prior to a cumulative dose of 4000 rads developed metastases or were dead of metastases at an average of 2 months after therapy. T cell levels in patients irradiated for esophageal carcinoma declined similar to bronchogenic carcinoma nonresponders: In all patients followed serially after therapy the levels were < 400 and all died within 6 months. In patients with either bronchogenic or esophageal carcinoma first studied after radiation therapy, T cell levels declined prior to or with the appearance of metastases. The data show a correlation between T cell levels and clinical tumor status in these patients. Monitoring of T cell levels thus may provide criteria for predicting response to radiation therapy and prognosis after treatment, and may be useful for the earlier selection of patients who may benefit from adjuvants such as chemotherapy or immunotherapy.

Immunobiology of skin cancer was studied with T cell levels with lymphocytic infiltration (LI) of the tumor (an *in vivo* measure of host-tumor relationship). T cell levels in preoperative patients with squamous (SCC) and basal (BCC) cell carcinoma were significantly lower than the noncancer control population (normals). T cell levels were significantly lower in patients with large tumors than in those with small tumors. T cell levels were significantly low in patients cured of large tumors, but were normal in those cured of small tumors. Patients with Bowen's disease not only had T cell levels significantly lower than normal as a group, but also had a significant increase in the number of patients with T cell levels less than 2 standard deviations below the normal mean, which may place them at greater risk for developing a second malignancy. There was a direct correlation between the degree of lymphocytic infiltration of the tumor, tumor size, and T cell levels: Small, well-localized tumors had a marked LI and high T cell levels, while the large, deeply invasive tumors had a minimal or absent LI and low T cell levels.

Immune competence was evaluated in cured patients who had been treated by irradiation for carcinoma of the laryngopharynx and compared with that of similar patients treated by surgery alone and normal controls. Cellular immunity was determined by quantitation of *in vitro* PHA-induced lymphocyte reactivity and peripheral blood T cell levels. Humoral immunity was assessed by measurement of serum immunoglobulin levels and by the effect of serum on *in vitro* normal lymphocyte reactivity to PHA. In 21 patients who were studied 4-23 years (mean 8.4) after surgical treatment alone for laryngopharyngeal carcinoma, neither cellular nor humoral immunity differed from that of 44 controls. In contrast, 14 patients who had been irradiated and subsequently cured 4-15 years (mean 9.0) prior to evaluation displayed significantly impaired cellular immune competence when compared to normals and patients treated by surgery alone. Since previous determinations of the effects of radiation therapy have been in patients who received irradiation to the thymic region or large area of bone marrow, this study documents for the first time that radiation therapy for cancer administered via portals that encompass a minimal area of the immune system may be associated with prolonged impairment of cellular immunity.

Studies of cellular immune competence in urologic cancer patients were performed using assays for the acquisition of delayed hypersensitivity to dinitrochlorobenzene (DNCB), the proliferative response of lymphocytes to phytohemagglutinin (PHA), and E rosette-forming lymphocytes. Vigorous cellular immunity was observed in bladder cancer patients with early lesions, well-differentiated lesions, and those without demonstrable vascular or lymphatic permeation by tumor cells, while impaired immune responsiveness was demonstrated in patients with advanced lesions, undifferentiated lesions, or those with demonstrable vascular of lymphatic permeation by tumor. A similar relationship between tumor stage and immunologic competence was observed among renal cancer patients. By contrast, impaired immunologic reactivity was found with similar frequencies among patients with early prostatic cancer and those with advanced disease. Cellular immune competence remained intact in testis tumor patients in the absence of general debility. Sera from prostatic cancer patients, but not other urologic cancer patients, exerted an inhibitory influence on PHA-stimulated lymphocyte blastogenesis. Increased ribonuclease (S-RNase) (an enzyme with immunosuppressive properties) activity was detected in sera from patients representing a variety of urologic malignancies. Moreover, a significant correlation was demonstrated between S-RNase and cutaneous reactivity to DNCB in these patients. Studies of E rosette-forming lymphocytes in patients irradiated for urologic cancer disclosed that such treatment produced a 50% reduction in the number of circulating lymphocytes and more than a 68% reduction in the T lymphocyte moiety, a reduction which persisted for up to two years.

Skin testing of patients with squamous cervical cancer or lung squamous carcinoma reveals a tumor specific reaction to the tumor associated antigens prepared from tumor cells. Ongoing studies of skin test reactivity in melanoma, breast, squamous carcinoma and sarcoma patients are being correlated with clinical course of the patients and with other in vitro immunological tests on these patients. Hopefully, this will result in a useful technique for the serial monitoring of patients before and after surgery, to assist diagnosis and prognosis.

The demonstration of the value of total parenteral nutrition in chronically ill or malnourished patients has led to the adoption of a total parenteral nutrition registrar and the standardization of this new technique to surgical patients who have undergone major abdominal operations. As a result, only one noninfectious complication of subclavian catheter insertion has occurred since July 1972. Infectious complications, which occurred in significant incidence before July 1972 with several episodes of candidemia and bacterial septicemias, have not occurred since then. In addition, a thorough metabolic study with nitrogen balances and electrolyte balances has been conducted on each patient, which allows the study of the metabolic changes with starvation and parenteral nutrition, as well as making hyperalimentation safer for critically ill patients.

The Branch has investigated the relationship of surgical stress and coagulation activation upon (1) the development of postoperative venous thrombosis; (2) the interaction of the coagulation mechanism and kinin generation in the production of the postoperative inflammatory response; and (3) the development of metastases. Template bleeding time, glass bead adhesion, and platelet factor 3 assays demonstrate in vivo and in vitro that two periods of platelet hyperactivity exist, namely on the second and eighth to tenth postoperative days. This correlates with the period of greatest risk of postoperative thromboembolic complications. The noninvasive bedside technique of impedance rheography has been used to detect both symptomatic and asymptomatic venous occlusions, and in the upper extremities to detect occlusions secondary to central venous catheterization. The data show that Hageman factor is activated intraoperatively within the first two hours of the onset of surgery. This observation supports the concept that the intrinsic coagulation system is activated by surgical stress, but places the time of activation earlier than previously thought, at the time of onset of trauma. Assays of bradykinin activity in biological fluid are difficult to interpret since the naturally occurring kininases so greatly abbreviate the life of generated bradykinin. Significant activation and utilization of the kinin precursors, pre-kallikrein and kininogen, begins intraoperatively and peaks on about the first postoperative day.

Previous work by the Branch has suggested that intraoperative infusions of large amounts of stored, bank blood may be an etiologic factor in producing postoperative pulmonary insufficiency. We have instituted clinical trials with blood filtration and studied the feasibility of intraoperative allotransfusion.

Our studies have demonstrated a high degree of microaggregate formation in units of bank blood. As a result, the Pall Ultipore filter is now used on intraoperative blood administration sets. Intraoperative allotransfusion has been evaluated as an alternative to large amounts of bank blood. In intact animals dead space, V_D/V_T, alveolar-arterial gradients, percent venous admixture, mean pulmonary arterial pressure, and pulmonary vascular resistance were significantly but transiently elevated. The effects of allotransfusion upon blood chemistry and the integrity of formed blood elements were examined patients undergoing surgery. Evidence of severe hemolysis was found in allotransfused blood with markedly elevated levels of plasma free hemoglobin, SGOT, LDH and CPK; and diminished hematocrit, hemoglobin, and erythrocyte count. Microaggregation was found in both bank and allotransfused blood. Contrary to the acidotic pH of bank blood, the mean pH of allotransfused blood was 7.72. Further refinements in pump design are required before routine human use if feasible. An in situ lung perfusion model in dogs was used to examine the pulmonary effects of autotransfused blood as compared with fresh and stored blood. The majority of animals perfused with stored blood had microscopic evidence of interstitial pulmonary edema, interstitial hemorrhage, intraalveolar fluid and alveolar congestion. Significantly, fewer animals showed these changes when they were perfused with autotransfused fresh blood. Using a primate lung model, fresh blood and old blood were transfused demonstrating that pulmonary injury does result from stored blood and that it may be mediated to some extent by the plasma fraction of the old stored blood.

In cooperation with the Clinical Center Diagnostic Radiology Department and the Metabolic Disease Section of the National Institute of Arthritis, Metabolism, and Digestive Diseases, studies have continued on developing techniques for localizing parathyroid adenomas by selectively catheterizing thyroid veins and radioimmunoassay of PTH. These techniques have proved to be of unique value, clinically, particularly in localization determinations as part of re-exploration procedures for parathyroid adenomas. An experimental study was conducted to determine the environmental factors affecting the function of transplanted parathyroid tissue. It was found that 12 hours of room temperature or drying for three hours at room temperature failed to affect the function of parathyroid glands as compared to fresh parathyroid glands which had been transplanted into hypercalcemic Fisher rats. Additionally, it was found that long-term cryopreservation at -196° C for nine months to a year functionally and histologically failed to affect the parathyroid tissue when compared to fresh, normal parathyroid gland. A microthermocouple probe which enables the investigator to record gradient temperature measurements underwent extensive evaluation in various animal models. Its usefulness and extreme accuracy will permit a more controllable application of the cryolesion. Additionally, the biological effects of cryodestruction on normal tissues, chiefly peripheral nerves, was evaluated in the dog and the monkey. After placement of the cryolesion on the facial nerve in the primate, complete facial paralysis was observed. Over a period of six to eight weeks, animals regained facial musculature function, in 100% of cases. Similar observations were made on the recurrent laryngeal nerve of the dog with interruption of vocal cord function and subsequent return of that function as the nerve regeneration took place. Work is underway at this time to determine the effects of cryosurgery on vascular structures, namely the primate carotid artery after 5000 rads of radiation. Initial studies have shown this type of injury to a large vascular structure free of expected complications. The effect of cryodestruction on the canine ureter is also under evaluation. Immunological work has continued on the evaluation of in situ oxide for the primary treatment of primary tumor specific therapy showing that there does appear to be a tumor specific immunity following cryonecrosis as compared to local excision and ligation. Additionally, in several animal tumor systems, cytotoxicity tests have shown a superior immunological effect associated with cryodestruction.

Using the I^{125} iododeoxyuridine (IDU) labelling technique developed by the Branch, host immunity to growing antigenic sarcomas was studied in a methylcholanthrene-induced guinea pig tumor system and in patients with skeletal and soft tissue sarcomas. Tumor-specific cellular immunity was assayed by cell-mediated cytotoxicity. Sera from animals and patients with growing sarcomas were evaluated for specific inhibition of this cellular immunity. The degree of tumor-specific cellular immunity in tumor-bearing animals and tumor-free animals was similar. However, sera from animals with palpable tumors inhibited specific cellular cytotoxicity. Sera drawn two weeks after tumor removal were noninhibitory. These data suggest that inhibition is mediated by enhancing antibody. Similarly, human sarcoma-specific cellular cytotoxicity was present in most sarcoma-bearing patients studied. Sera from 14 out of 16 sarcoma-bearing patients inhibited sarcoma-specific cellular immunity, but did not inhibit cellular immunity against carcinoma. Serum inhibition correlated with the clinical stage of the disease. These data demonstrate an immunological phenomenon which may be related to the finding of progressive sarcoma growth in the presence of tumor-specific cell-mediated immunity.

A humoral assay was developed in which I^{125} IDU was used as a tumor cell label. This microassay was developed to detect cytotoxic tumor-specific antibody against I^{125} IDU labelled sarcoma cells in mice. The technique uses small amounts of serum and cells and is simple, sensitive, and objective. The cytotoxicity as determined by radioactive counting of cells using this technique correlates well with cytotoxicity as determined by visual counting.

A simplified isotopic antiglobulin assay was developed for the study of tumor-specific antibody. This test is similar to the indirect immunofluorescence test. Both test target cells against immune serum which is then tagged with a reagent that attaches to the immune serum. In the indirect immunofluorescence test, this reagent is a fluoroscein-labelled antiglobulin, but in the isotopic antiglobulin test the antiglobulin is labelled with I^{125}. Previously, this test has been performed in suspension. The current modifications allow the test to be performed with plated tissue culture monolayers and using a simplified washing procedure, the result being a rapid and sensitive technique for detecting antibody against tumor-specific antigens.

The Branch has investigated cross-reactions between fetal antigens and tumor antigens in experimental animal tumors utilizing the guinea pig, rat, and mouse. Immunization of animals to second trimester (allogenic or syngenic) fetal tissue affords some degree of protection compared to control animals immunized to allogenic adult tissue when challenged with various chemically-induced tumors. Although some resistance to tumor challenge has been shown in femal exbreeder animals following multiple pregnancies, this has not been a consistent finding in all three animal systems. In addition, guinea pigs and rats were immunized to second trimester syngenic fetal tissue or chemically-induced syngenic tumors and subsequently skin tested with cell membrane or soluble antigen extracts of the tumors and found to show positive skin tests to the tumor antigen in both groups of animals, those immunized against the tumor and those immunized against the second trimester fetal tissue.

The effect of immunization of Balb/c mice with BCG vaccine on the subsequent development of melanoma following intramuscular challenge with Cloudman melanoma cells has been studied. Seven weeks following tumor challenge the overall tumor incidence in the BCG immune group was 34% lower than in the control group. The decreased tumor incidence in the BCG immune animals may have resulted from either nonspecific and new stimulation due to BCG immunization, or from specific rejection of melanoma cells due to sharing of common antigens by tubercle bacilli and melanoma. In vitro studies are underway to investigate these possibilities. Studies of preferential metastases have been carried out utilizing a spontaneously arising reticulum cell sarcoma in C_3H mice which selectively metastasizes to the spleen. Placement of these animals in parabiosis has confirmed the strong splenotropic tendency of these metastases. Study of this tumor in parabiotic animals provides a model which will permit evaluation of the role of immunologic factors in preferential tumor metastases. Studies of the effect of parabiosis on tumor growth rate carried out previously in the Branch have been confirmed and extended. Placement of a tumor-bearing animal in parabiosis in the normal animal results in inhibition of tumor growth rate. This is found not due to nonspecific factors such as surgery, confinement, or restraint. Recruitment of the immune response of the normal and immune animals joined in parabiosis with tumor-bearing animals may be responsible for the decreased tumor growth rate in the tumor-bearing animals. Studies to elucidate the mechanism of this effect are in progress.

A pilot study is underway to evaluate the affinity of isotopically labeled Bleomycin for primary and metastatic neoplasms via total body rectilinear scanning and regional gammagraphic examination. It has been planned that through its interaction with DNA, Bleomycin might aid in localizing clinically unsuspected metastatic disease.

Presently, the Urology Service is involved in several laboratory and clinical investigations basic to genitourinary cancers. These projects and protocols are mostly related to the host resistant immunology, cytogenetics, electron-microscopy studies and development of new techniques of fetal surgery in understanding the pathogenesis of congenital anomalies. The laboratory studies have been conducted on: 1) induction of embryonal carcinoma of the testis by genital ridge tranplantation in syngenic mice and the studying of the pathogenesis, various modalities of chemotherapy and immunotherapy; 2) carcinogenic effect of FANFT on the bladder and immunologic changes before and after the development of bladder cancer in mice; 3) cross-reactivity of fetal antigen with induced bladder, prostate and testis cancers in rodents; 4) immunotherapy of experimental bladder and testis cancers with BCG, vibrio cholera neuraminidase (VCN) and radiated cancer cell alone or as a combination with appropriate controls; 5) fetal surgery in understanding the pathogenesis of congenital anomalies which are more common in the genitourinary tract than in any other organ system; 6) the effect of radiation and radiomemic chemotherapeutic agents such as the effect of cyclophosphamide on urothelium to clarify the mechanism of fatal massive hematuria as the complication of these anticancer drugs.

The clinical program entails new surgical technique for treatment of recurrent persistent vesicovaginal fistula refractory to any conventional treatment. The study of the urological aspects of the genitourinary cancers with electron-microscopy and immunologic methods. We have been able to show the association of an RNA virus with transitional cell carcinoma of the renal pelvis. C particles have been identified in experimentally induced embryonal carcinoma in mice. Massive life-threatening hematuria which is secondary to radiation or cyclophosphate therapy has been controlled by a newly developed S.B. technique of instillation of refined formalin in the bladder to stop severe bleeding. Studies are underway to determine whether human testis tumors might have useful tumor markers, such as chorionic gonadotropin, placental, lactogen, oncogetoprotein, placental alkaline phosphatase and to evaluate the immunologic aspects of these tumors both in vitro and in vivo.

TABLE 1

Individual Written Intramural Consultations Received by Surgery Branch, NCI
Listed by Institutes
1963-1973

	NCI	NINDS	NIAMD	NHLI	NIAID	NIMH	NICHD	NIDR	NEI	EHS	TOTAL
1963	366	184	221	228	158	37	-	26	-	-	1220
1964	356	121	207	201	104	33	-	24	-	-	1046
1965	429	129	216	277	119	39	-	31	-	-	1240
1966	362	271	201	197	86	59	-	11	-	7	1205
1967	343	215	245	166	85	61	-	17	-	-	1132
1968	278	143	192	203	111	54	-	25	-	7	1013
1969	314	131	248	207	128	45	-	31	-	10	1114
1970	199	59	137	104	96	41	-	12	-	7	655
1971	220	98	152	89	94	62	15	28	28	18	804
1972	189	72	134	71	98	58	8	15	10	14	669
1973	203	68	151	82	87	51	9	17	16	17	701

TABLE 2

Summary of Written Extramural Consultations Requested by or Through Surgery Branch, NCI

1963-1973

	ENT	General Surgery	Gynecology	Urology	Oncology	Total
1963	346	14	7	18	9	394
1964	233	6	4	4	3	242
1965	268	6	2	9	1	286
1966	271	8	4	13	2	298
1967	294	11	1	1	1	308
1968	306	8	1	1	1	317
1969	329	8	2	3	2	344
1970	778	12	2	2	0	794
1971	704	3	0	0	0	707
1972	1034	20	0	0	0	1054
1973	1110	21	0	0	0	1131

TABLE 3

Annual Report of NIH Surgical Suites
Listing the Number of Major & Minor Procedures
Carried Out by Each of the Operative Units at NIH
April 1, 1973 - March 31, 1974

Patient		NCI	NHLI	NINDS		NIAID	NIAMD	NEI	NIMH	NICHD	NIDR	CONSULTANTS	Total #Pt. Operated	
				Neuro.	Muscle									
NCI	Major	224	21	20	-	-	-	-	-	-	-	19	284	768
	Minor	456	6	11	3	-	-	-	-	-	-	8	484	
NHLI	Major	14	199	1	-	-	-	-	-	-	-	1	215	265
	Minor	15	29	-	3	-	-	-	-	-	-	3	50	
NINDS	Major	3	2	67	-	-	-	-	-	-	-	5	77	309
	Minor	11	2	10	205	-	-	1	1	-	1	1	232	
NIAMD	Major	26	5	2	-	-	-	-	-	-	-	8	41	74
	Minor	17	-	-	8	-	-	-	-	-	-	8	33	
NIAID	Major	7	1	6	-	-	-	-	-	-	-	2	16	68
	Minor	31	1	1	2	-	-	-	-	-	1	16	52	
NIDR	Major	-	-	-	-	-	-	-	-	-	-	-	0	1
	Minor	-	-	-	-	-	-	-	-	-	1	-	1	
NIMH	Major	-	-	-	-	-	-	-	-	-	-	-	0	1
	Minor	3	-	1	-	-	-	-	-	-	-	-	4	
NICHD	Major	2	1	3	-	-	-	-	-	-	-	-	6	17
	Minor	8	-	3	-	-	-	-	-	-	-	-	11	
NEI	Major	-	-	-	-	-	-	57	5	-	-	-	62	78
	Minor	1	1	-	1	-	-	13	-	-	-	-	16	

TABLE 3 (CONTINUED)

MAJOR TOTAL	276	229	93	0	0	0	57	5	0	0	35	695	1584
MINOR TOTAL	542	39	32	222	0	0	14	1	0	3	36	888	
GRAND TOTAL	818	268	125	222	0	0	71	6	0	3	71	1584*	

*Of these 1584 cases, 107 patients were on OPD status.

Cardiac Cath. Lab. procedures 10A2 - 520
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TABLE 4

Number of Surgical Procedures Performed by NCI Surgery Branch Staff and Magnitude of the Procedures in 1973
April 1, 1973 - March 31, 1974

	10 East	Other NCI	Other Institutes	Total
1963	470	159	399	1028
1964	424	215	256	895
1965	394	112	199	705
1966	410	113	218	741
1967	440	145	140	725
1968	394	205	113	712
1969	339	233	158	730
1970	301	273	230	804
1971	228	273	179	680
1972	307	146	136	589
1973	328	263	227	818
Major	192	152	82	426
Minor	136	111	145	392

Serial No. NCI-3800
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies

Previous Serial Number: Same

Principal Investigators: Entire Staff of the Surgery Branch, NCI

Other Investigators: G.D. Aurbach, M.D., P.P. Carbone, M.D., V.T. DeVita, M.D., R.E. Johnson, M.D., F.C. Bartter, M.D., L.B. Thomas, M.D., R.D. Powell, M.D., J.L. Ziegler, M.D. and J.L. Doppman, M.D.

Cooperating Units: Metabolic Disease, NIAMD; Medical Oncology, NCI; Medicine Branch, NCI; Radiation Branch, NCI; Endocrinology Branch, NHLI; Laboratory of Pathology, NCI; Pediatric Oncology Branch, NCI; and Department of Diagnostic Radiology, Clinical Center

Man Years:

Total:	6-1/2
Professional:	4
Other:	2-1/2

Project Description:

Part I. Service Aspects

Part II. Specific Cooperative Studies

A. Hyperparathyroidism

B. Thyroid Carcinoma

C. Lymphoma and Hodgkin's Disease

D. Splenectomy and its Role in Chronic Myelogenous Leukemia

Project Description:

Part I. Service Aspects

Objectives:

The object of this project is to make available human tissue for histologic, histochemical, biochemical, biophysical, viral and immunological studies to scientists working in various Branches of the National Cancer Institute and other Institutes who require tissue for studies. As a rule, such studies are not done as cooperative projects. In addition, it implies furnishing general surgical consultative coverage of patients admitted to other Branches of the National Cancer Institute and/or other Institutes and assisting with their study projects. This surgery is often definitive in nature, the therapy regime requiring surgical tissue excision as the sole type of therapy, or more often as an adjuvant to other modes of treatment.

Methods Employed:

Methods employed are standard surgical procedures. Material from this project is obtained mostly from patients hospitalized for study in other Branches or Institutes. Occasionally, it is necessary to admit patients for specific studies in which a specific type of tissue is obtained. Tumor tissue, lymph nodes, muscle, skin and liver and spleen comprise the greater share of tissue removed.

Major Findings:

In that the major portion of the work involved in this project is done as a surgical consultative service and not as a cooperative study, the project results are not immediately available to the surgical service. During the course of supplying this service, 669 individual requests for consultation were received. These figures do not include the extensive follow-up which is afforded each of these consultations, but only the original written request to participate in their care. Of these requests, 282 resulted in an operative procedure, 35% of which entailed major surgery. See attached Tables 1-4.

Significance to Biomedical Research and Program of the Institute:

This project is a service function for other Branches of the National Cancer Institute and National Institutes of Health. It is seldom that the Surgery Branch is included in the research studies other than supplying the tissue to the investigator; however, this trend is being reversed and several studies are being carried out on a cooperative basis with Surgery Branch Staff now serving as active counselors in the overall study.

Proposed Course:

Continued cooperative assistance within the physical limits of the Surgery Branch Staff.

Project Description:

Part II. Specific Cooperative Studies

A. Hyperparathyroidism

Objectives:

The Clinical Endocrinology Branch, NHLI, the Section on Mineral Metabolism, Metabolic Diseases Branch, NIAMD, and the Diagnostic Radiology Branch, Clinical Center, have programs underway to determine clinical, laboratory and radiographic criteria which justify surgical exploration of the neck for the treatment of parathyroid disease. This joint program is expected to increase the reliability of diagnosis and efficacy of treatment for parathyroid disorders, particularly in early cases where the treatment will reduce the incidence of secondary renal disease.

Methods Employed:

Standard surgical and anesthetic procedures are used for operating on the patients. In addition to the customary extensive clinical evaluation and laboratory determinations of static derangements of calcium and phosphorus metabolism, a battery of tests are performed to measure responses to induced distortions of calcium and phosphorus hemostasis. Additionally, selective inferior thyroid arteriographic studies are done preoperatively, as is selective venography and removal of blood samples from various veins draining the parathyroid complex identified by venography. Radioimmunoassay determinations of parathyroid hormone are made upon the venous samples so obtained. At operation, prior to any exterpative procedure, intensive efforts are made to identify positively by frozen section all parathyroids present.

Major Findings:

Selective inferior thyroid arteriography continues to be of considerable value in the preoperative localization of abnormal parathyroid glands, particularly when the subtraction technique is applied to the arteriograms. Selective venography has not been helpful in finding abnormal parathyroid glands, but has enabled the radiographer to identify the appropriate veins from which to withdraw blood for parathyroid hormone assays. The parathyroid hormone assays have been extremely encouraging in that out of 31 patients studied, the lateralization of the parathyroid neoplasm has been successfully predicted in all 30 cases. This technique is extremely helpful in patients in which adenoma was not found at the first surgery.

Significance to Biomedical Research and Program of the Institute:

Early diagnosis and removal of overactive parathyroid tissue is an important step toward preservation of renal function from the ravages of stone formation. The addition of selective venous catheterization and radioimmunoassay for parathyroid hormonal levels of samples thereby obtained is an important tool for both diagnosis of primary hyperparathyroidism and preoperative localization of the abnormal tissue. Selected angiography has continued to bear out its earlier promise as an important aid to the surgeon in finding the abnormal glands readily, with a resultant decrease in operating time and morbidity.

Proposed Course:

Continued cooperative studies.

Project Description:

Part II. Specific Cooperative Studies

B. Thyroid Carcinoma

Objectives:

To assess the clinical, pathological and endocrinological parameters of patients with Sipple's syndrome (medullary carcinoma of the thyroid gland, pheochromocytoma and parathyroid hyperplasia).

Methods Employed:

Presently, a large number of patients have been admitted to the NIH with the diagnosis of Sipple's syndrome. These patients have been diagnosed positively by the presence of an elevated thyrocalcitonin level in their peripheral blood. Patients with peripheral levels above 0.3 nanograms/ml. can be diagnosed with 100% accuracy as having medullary carcinoma of the thyroid gland. A large number of these patients have concurrent pheochromocytomas and parathyroid hyperplasia. Large numbers of this family have been screened and admitted to the NIH for study. The disease status is diagnosed and they subsequently undergo operation for removal of the thyroid carcinoma and other disease organs should they be manifest.

Major Findings:

The elevated peripheral level of thyrocalcitonin has been an excellent screening test for the presence of medullary carcinoma of the thyroid gland. We have had no false positive findings in that all patients with levels above 0.3 nanograms/ml. have thyroid carcinoma. At present, of all of the serological tests for the detection of cancer, this one has the greatest accuracy. It has also been found that histaminase levels are elevated once the disease metastasizes outside of the thyroid gland. This is a good index of measuring extent of disease in those patients who either have undergone surgery or in those who have recurred or who have residual tumor left behind following surgery. The standard operative procedure that has been employed in these patients has been a total thyroidectomy with removal of the central nodes of the neck. Most of the patients in our group have had generalized parathyroid hyperplasia and have undergone subtotal parathyroidectomy.

Significance to Biomedical Research and Program of the Institute:

This methodology has been extremely important in that the serological screening test has had a high degree of accuracy in the thyroid carcinoma. This allows the early diagnosis of medullary carcinoma of the thyroid gland and hopefully total removal of the neoplasm before metastasis has occurred.

Proposed Course:

To screen the remainder of the pedigree and admit those diseased for study and surgery.

Project Description:

Part II. Specific Cooperative Studies

C. Lymphoma and Hodgkin's Disease

Objectives:

To assess the adequacy of staging of patients with lymphoma and Hodgkin's disease by nonsurgical methods. To assess the effect of splenectomy on patient survival and tolerance of radiotherapy and chemotherapy treatment regimens.

Methods Employed:

All patients admitted to the Chemotherapy and Radiotherapy Services with lymphoma and Hodgkin's disease undergo routine staging by the combined evaluation of clinical and physical findings, liver chemistries, lymphangiograms, percutaneous liver biopsy, peritoneoscopy, liver biopsy and chest x-ray. To assess the accuracy of these staging techniques, selected patients have been subjected to laparotomy and biopsy of the liver and abdominal lymph nodes. Selected adenopathy detected after treatment is further evaluated by biopsy and histologic examination.

Major Findings:

The mounting experience gained through the examination of tissues removed at laparotomy in patients with lymphoma and Hodgkin's disease in comparison with staging data will more accurately delineate those patients who will profit from the inconvenience of laparotomy. Sufficient evidence has accumulated to allow the conclusion that all patients will not benefit from staging laparotomy, a conclusion that is at variance with the findings of several other major treatment centers. On the other hand, biopsy of multiple abdominal nodal areas has demonstrated the limitations of lymphangiography in assessing the extent of abdominal nodal disease. The contribution made by abdominal laparotomy and biopsy, however, is clearly dependent on the treatment proposed and how it is altered by the finding of unsuspected positive abdominal lymph nodes.

A sequential evaluation of the liver by percutaneous biopsy, followed by peritoneoscopy-biopsy in patients with negative percutaneous biopsies, shows that the second biopsy averts open biopsy in a significant incidence of patients. For instance, in non-Hodgkin's lymphomas, only 10 of 24 consecutive patients required open biopsy, because in 10 patients, positive tissue was obtained by percutaneous biopsy, and in 4 additional patients, biopsy with peritoneoscopic control yielded positive tissue.

The evidence emerging from other treatment centers that splenectomy favorably affects patient survival and tolerance or radiotherpay and chemotherapy in Hodgkin's disease and lymphosarcoma is currently being assessed at this institution by a prospective study of the effects of splenectomy on these parameters. Although the value of splenectomy in those patients with abnormal consumption of blood cells is continuously being demonstrated as is the laboratory benefit of splenectomy and the salutory benefit of removal of spleens unresponsive to chemotherapy and radiotherapy, sufficient data has not yet accumulated to allow assessment of the effects of splenectomy on tolerance of subsequent specific treatment and overall survival.

Significance to Biomedical Research and Program of the Institute:

The cooperation of the Surgery Branch with the divisions of the Institute concerned with treatment of patients with lymphoma and Hodgkin's disease results in a continuously improving program of diagnosis and treatment of these patients. These studies allow the intelligent selection of those patients who would benefit by laparotomy prior to specific treatment of the disorder.

Proposed Course:

Studies in progress will correlate laparotomy findings in these diseases with response to therapy and long-term prognosis. The effect of splenectomy on tolerance to chemotherapy and radiotherapy will be determined.

Project Description:

Part II. Specific Cooperative Studies

D. Splenectomy and Its Role in Chronic Myelogenous Leukemia

Objectives:

To reassess the effect of splenectomy on normalization of blood cell kinetics in patients with chronic myelogenous leukemia (CML); to delineate those patients that will benefit from splenectomy.

Methods Employed:

Thrombocytopenia is usually a late manifestation of CML. Most often it is associated with transformation of the disease to the blastic phase. In a smaller number of patients, thrombocytopenia, which may be a direct manifestation of the disease process or result from an inordinate sensitivity to the myelosuppressive effects of chemotherapy, occurs in the chronic stage. This latter instance severely limits the effective treatment of the disease and may require hospitalization and platelet transfusion support. In patients presenting with this clinical picture, splenectomy is performed. The effects of this operative procedure on the subsequent hematologic picture and tolerance to therapy is evaluated.

Major Findings:

The indications for splenectomy in this group of patients fell into two general categories: (1) The inability to control the clinical and hematological manifestations of the disease because of an inordinate sensitivity of platelet blood counts to busulphan; and (2) prolonged pancytopenia and severe thrombocytopenia secondary to busulphan myelosuppression, resulting in frequent hospitalization for multiple platelet transfusions. All patients tolerated the operation without excessive bleeding. Platelet concentrate transfusions were utilized throughout the procedure and during the postoperative period. There was an almost immediate rise in the platelet count within 48 hours in all patients. The extent of postoperative thrombocytosis did not appear to correlate with spleen size at the time of surgery. In one case, the spleen histology predicted blastic transformation, showing extensive replacement of the spleen by immature blast cells. In the other cases, the histopathology was compatible with CML or myeloid metaplasia. There appeared to be no correlation between the numbers of megakaryocytes in the marrow and subsequent response to therapy. The postoperative rise in platelet count was rapid in most patients. In all patients with hemorrhagic manifestations of thrombocytopenia, splenectomy ameliorated the bleeding and obviated the need for further platelet transfusion.

Significance to Biomedical Research and Program of the Institute:

This study delineates a role for splenectomy for the treatment of CML. Those patients with prolonged thrombocytopenia and hemorrhagic manifestations due to busulphan-related myelosuppression unresponsive to corticosteroids and frequent platelet transfusions and those with inordinate platelet sensitivity to chemotherapy are candidates for splenectomy. The rapid rise in platelet count and cessation of hemorrhagic complications were gratifying results in these patients. This information is of critical importance to all involved in the exploration of improved therapeutic approaches to the treatment of this disease.

Proposed Course:

This project will continue, with further exploration of the beneficial effect of splenectomy on the course of patient with CML. These include exploration of less obvious effects, including immunologic alterations, precipitated by splenectomy.

TABLE 1
Individual Written Intramural Consultations Received by Surgery Branch, NCI Listed by Institutes 1963-1973

	NCI	NINDS	NIAMD	NHLI	NIAID	NIMH	NICHD	NIDR	NEI	EHS	TOTAL
1963	366	184	221	228	158	37	-	26	-	-	1220
1964	356	121	207	201	104	33	-	24	-	-	1046
1965	429	129	216	277	119	39	-	31	-	-	1240
1966	362	271	201	197	86	59	-	11	-	7	1205
1967	343	215	245	166	85	61	-	17	-	-	1132
1968	278	143	192	203	111	54	-	25	-	7	1013
1969	314	131	248	207	128	45	-	31	-	10	1114
1970	199	59	137	104	96	41	-	12	-	7	655
1971	220	98	152	89	94	62	15	28	28	18	804
1972	189	72	134	71	98	58	8	15	10	14	669
1973	203	68	151	82	87	51	9	17	16	17	701

TABLE 2
Summary of Written Extramural Consultations Requested by or Through Surgery Branch, NCI
1963-1973

	ENT	General Surgery	Gynecology	Urology	Oncology	Total
1963	346	14	7	18	9	394
1964	233	6	4	4	3	242
1965	268	6	2	9	1	286
1966	271	8	4	13	2	298
1967	294	11	1	1	1	308
1968	306	8	1	1	1	317
1969	329	8	2	3	2	344
1970	778	12	2	2	0	794
1971	704	3	0	0	0	707
1972	1034	20	0	0	0	1054
1973	1110	21	0	0	0	1131

TABLE 3

Annual Report of NIH Surgical Suites
Listing the Number of Major & Minor Procedures
Carried Out by Each of the Operative Units at NIH
April 1, 1973 - March 31, 1974

Patient		NCI	NHLI	NINDS		NIAID	NIAMD	NEI	NIMH	NICHD	NIDR	CONSULTANTS	Total #Pt. Operated	
				Neuro.	Muscle									
NCI	Major	224	21	20	-	-	-	-	-	-	-	19	284	768
	Minor	456	6	11	3	-	-	-	-	-	-	8	484	
NHLI	Major	14	199	1	-	-	-	-	-	-	-	1	215	265
	Minor	15	29	-	3	-	-	-	-	-	-	3	50	
NINDS	Major	3	2	67	-	-	-	-	-	-	-	5	77	309
	Minor	11	2	10	205	-	-	1	1	-	1	1	232	
NIAMD	Major	26	5	2	-	-	-	-	-	-	-	8	41	74
	Minor	17	-	-	8	-	-	-	-	-	-	8	33	
NIAID	Major	7	1	6	-	-	-	-	-	-	-	2	16	68
	Minor	31	1	1	2	-	-	-	-	-	1	16	52	
NIDR	Major	-	-	-	-	-	-	-	-	-	-	-	0	1
	Minor	-	-	-	-	-	-	-	-	-	1	-	1	
NIMH	Major	-	-	-	-	-	-	-	-	-	-	-	0	1
	Minor	3	-	1	-	-	-	-	-	-	-	-	4	
NICHD	Major	2	1	3	-	-	-	-	-	-	-	-	6	17
	Minor	8	-	3	-	-	-	-	-	-	-	-	11	
NEI	Major	-	-	-	-	-	-	57	5	-	-	-	62	78
	Minor	1	1	-	1	-	-	13	-	-	-	-	16	

TABLE 3 (CONTINUED)

MAJOR TOTAL	276	229	93	0	0	0	57	5	0	0	35	695	1584
MINOR TOTAL	542	39	32	222	0	0	14	1	0	3	36	888	
GRAND TOTAL	818	268	125	222	0	0	71	6	0	3	71	1584*	

*Of these 1584 cases, 107 patients were on OPD status.

Cardiac Cath. Lab. procedures 10A2 - 520
" " " " 7N - 1
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TABLE 4

Number of Surgical Procedures Performed by NCI Surgery Branch Staff and Magnitude of the Procedures in 1973

April 1, 1973 - March 31, 1974

	10 East	Other NCI	Other Institutes	Total
1963	470	159	399	1028
1964	424	215	256	895
1965	394	112	199	705
1966	410	113	218	741
1967	440	145	140	725
1968	394	205	113	712
1969	339	233	158	730
1970	301	273	230	804
1971	228	273	179	680
1972	307	146	136	589
1973	328	263	227	818
Major	192	152	82	426
Minor	136	111	145	392

Publications:

Bilezikian, J.P., Pearson, K.D., Powell, D., Ketcham, A.S.. Doppman, J.L., and Aurbach, G.D.: Selective venous catheterization of the neck: Localization of a parathyroid adenoma drained anomalously by non-thyroidal veins. J. Clin. Endocrin. Metabol., 36: 826-829, 1973.

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In Press:

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Serial No. NCI-3801
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of Cancer Surgery as to its Effectiveness and Safety in Providing Definitive and Therapeutic Care of Cancer Patients

Previous Serial Number: Same

Principal Investigators: A.S. Ketcham, M.D., P.B. Chretien, M.D., and the entire Staff of the Surgery Branch, NCI

Other Investigators: L.B. Thomas, M.D., E.W. Chu, M.D.; W.E. Wright, M.D., J. Folio, M.D.; R.E. Johnson, M.D., T.C. Pomeroy, M.D.; G.S. Johnston, M.D.; J.L. Doppman, M.D., J.R.L. Herdt, M.D.; J.L. Ziegler, M.D.; V.T. DeVita, Jr., M.D., P. Carbone, M.D.; W.T. Terry, M.D.; J.B. Block, M.D.; R.B. Herberman, M.D.; J.D. MacLowry, M.D., H.R. Gralnick, M.D.; D.C. Tormey, M.D.; D.M. Fried, M.D.; and R.C. Hoye, M.D.

Cooperating Units: Laboratory of Pathology, NCI; Clinical Investigations and Research Services Branch, NIDR; Radiation Branch, NCI; Department of Nuclear Medicine, CC; Diagnostic Radiology Department, CC; Hematology and Supportive Care Branch, NCI; Medicine Branch, Division of Cancer Treatment, NCI; Immunology Branch, NCI; Office of the Director, CC; Laboratory of Cell Biology, NCI; Clinical Pathology Department, CC; Medical Breast Cancer Service, NCI; Rehabilitation Department, CC

Man Years:

Total:	8-1/2
Professional:	5
Other:	3-1/2

Project Description:

Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix

A. Surgical Treatment of Carcinoma of the Cervix

B. Scalene Nodes as Indicators of Disseminated Cancer

Part II. The Surgical Treatment of Melanoma

A. Surgical Staging of Melanoma Patients

B. Immunotherapy and Chemotherapy as Adjuvants to Surgical Treatment

C. Malignant Melanoma: Screening of Potential Surgical Candidates with ^{67}Gallium Scintography

Part III. Fine Needle Aspiration Biopsy in the Diagnosis of Solid Tumors

Part IV. Evaluation of Uretero-Ileo-Cutaneous Anastomosis Following Radical Surgery for Pelvic Cancer

Part V. Extended Deltopectoral Flap Reconstruction of Anterior Oral Defects

Part VI. A Study of the Results of Surgical Treatment and Effects of Adjunctive Therapy in Cancer of the Head and Neck

Part VII. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

A. Hemipelvectomy: Its Role in the Treatment of Extremity Sarcoma

B. The Use of Coumadin Anticoagulation as an Adjunct to the Surgical Treatment of Sarcomas

C. Medullary Extension of Osteosarcoma

Part VIII. An Improved Technique for Radical Groin Dissection

Part IX. The Role of Bacterial Infection in Cancer Surgery

Part X. Treatment of Breast Cancer

Part XI. Testis Cancer

Part XII. Use of ^{111}Indium Labelled Bleomycin in Evaluation of Neoplastic Disease

Project Description:

Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix

A. Surgical Treatment of Carcinoma of the Cervix

Objectives:

To evaluate extended surgical procedures for treatment of patients with recurrent cervical cancer and for selected patients with primary carcinoma of the uterine cervix.

Methods Employed:

Approximately 2100 patients with cancer of the uterine cervix have been referred to the Surgery Branch for evaluation. From this large group, 550 were admitted for inpatient evaluation, 406 of whom underwent abdominal exploration with plans for definitive surgical therapy.

Of the patients explored, 163 were not candidates for exenterative surgery because of the presence of extrapelvic lymph node metastasis, intraperitoneal spread, or disease fixed to the bony pelvis. Eighty-two patients had relatively early cancers and could be effectively managed with a radical hysterectomy and pelvic lymphadenectomy. Thirteen of these patients had small radiorecurrent lesions, the remainder had primary cervical cancer.

One hundred sixty-one patients required either partial or total pelvic exenteration for control of their disease.

Major Findings:

The major points of interest are summarized in the accompanying tables. The 5-year actuarial survival of those treated with radical hysterectomy and pelvic lymphadenectomy was 83.5%. Those patients with radiorecurrent or persistent disease survived at a rate similar to those with primary carcinoma. The presence of metastatic carcinoma in the pelvic lymph nodes adversely affected prognosis. The 5-year actuarial survival for those treated with exenteration of the pelvic viscera was 38%.

Significance to Biomedical Research and Program of the Institute:

The results obtained with extended surgical procedures are highly favorable and surpass those reported from other institutions (Annual Report on the Results of Treatment of Carcinoma of the Uterus and Vagina, 14th Volume). Extended surgical procedures are demonstrated to be effective in controlling advanced cervical cancer and are the only effective treatment modalities for patients with radiorecurrent cervical cancer. Selected individuals are offered vaginal reconstruction in an attempt to contribute to complete rehabilitation. The important ancillary studies generated by this program are detailed elsewhere in this report and include physiologic alterations following massive major surgery, dynamics of various forms of urinary diversion, the diagnostic advantages of "fine needle" cytology, and the efficacy of exfoliative cytology from urinary conduits in the early diagnosis of recurrent cancer.

TABLE I

CARCINOMA OF THE CERVIX: ACTUARIAL 5-YEAR SURVIVAL FOLLOWING TREATMENT WITH EXTENDED SURGICAL PROCEDURES

OPERATION PERFORMED	PRIMARY CARCINOMA PELVIC LYMPH NODES			RECURRENT CARCINOMA PELVIC LYMPH NODES		
	Positive	Negative	Total	Positive	Negative	Total
Radical Hysterectomy and Pelvic Lymphadenectomy	60.0%	91.3%	82.8% (69)*	85.7%	83.3%	84.6% (13)
Pelvic Exenterations (Partial and Total)	54.2%	52.2%	52.6% (66)	18.4%	35.6%	31.8% (95)
TOTAL (All extended surgical procedures without resection of bony pelvis)	56.4%	74.4%	67.6% (135)	30.5%	39.9%	39.1% (109)

*Numbers in parentheses represent the number of patients studied.

TABLE II

CORRELATION OF THE MEIGS' SURGICAL PATHOLOGIC STAGING WITH SURVIVAL OF 162 PATIENTS WITH CARCINOMA OF THE CERVIX WHO UNDERWENT PELVIC EXENTERATION

	5-YEAR CUMULATIVE PERCENT SURVIVAL		
PATHOLOGIC STAGING	PRIMARY CASES	RECURRENT CASES	ALL CASES
$A + A_o$	75% (5)*	43% (7)	60% (16)
B	56% (10)	0% (14)	33% (24)
C	31% (11)	49% (25)	44% (36)
D	60% (10)	17% (6)	44% (16)
E	43% (32)	18% (38)	30% (70)

*Numbers in parentheses represent total cases in each group.

TABLE III

PRIMARY CARCINOMA OF THE CERVIX: ACTUARIAL SURVIVAL (BY STAGE) FOLLOWING EXTENDED SURGICAL PROCEDURES

	PELVIC LYMPH NODES		
	Positive	Negative	Total
Stage I	75%	87%	84.3% (37)*
Stage II	60%	86%	80.0% (42)
Stage III	50%	75%	60.0% (10)
Stage IV	50%	32%	39.5% (37)

*Numbers in parentheses represent the number of patients studied.

Proposed Course:

The limits and the values of extended surgical procedures have been defined. Current plans call for a refinement of the diagnostic procedures to determine more specific and reliable criteria for operability and long-term cure. Although the Surgery Branch is no longer actively recruiting patients, thereby phasing out this project, significant numbers of patients with radiorecurrent disease are still being referred. These patients are included in the ancillary studies described above.

Project Description:

Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix

B. Scalene Nodes as Indicators of Disseminated Cancer

Objectives:

To determine the instance of tumor dissemination, from primary pelvic cancer, to the scalene node bearing areas.

Methods Employed:

All patients with biopsy proven cancer of the structures of the female pelvis who are candidates for definitive curative surgery will have left scalene node biopsy. The procedure is carried out only in the absence of distal tumor spread. A generous pad of lymph node bearing tissue from the left subclavicular fossa is obtained from microscopic analysis. Using local anesthesia, this minor operative procedure can be carried out without complications.

Major Findings:

Ninety-six patients with carcinoma of the cervix whose preoperative evaluation revealed no evidence of cancer spread beyond the confines of the pelvis and who were candidates for surgery had scalene lymph pad tissue removal. Twelve of these patients were found to have unsuspected metastases to one of the lymph nodes in the subclavicular fossa. None of these patients had any evidence of pulmonary or other metastases. They were, therefore, deemed incurable by surgery and a radical procedure avoided which would only have been palliative.

Significance to Biomedical Research and Program of the Institute:

A significant number of patients developed metastatic disease at such a time interval following primary tumor treatment that there is the suggestion that the metastases were present preoperatively. No matter how complete and extensive the pretreatment evaluation, the surgeon must face this distressing situation only too often. The identification of metastatic disease from pelvic carcinoma to unsuspected anatomical areas adds another preoperative modality of determining what patient may be free of cancer metastases at the time of definitive surgery.

Proposed Course:

Continue study of all patients admitted for treatment of primary pelvic disease to rule out previously unrecognized metastases.

Project Description:

Part II. The Surgical Treatment of Melanoma

A. Surgical Staging of Melanoma Patients

Objectives:

To evaluate the extent of occult disease in pathologic Stage II melanoma patients whose disease is presumably confined to the first regional lymph node area.

Methods Employed:

Patients with Stage II melanoma undergo a comprehensive Surgery Branch diagnostic work-up. In patients whose primary lesion is on the leg so that therapeutic laparotomy would be required as part of the Surgery Branch treatment for melanoma, intraoperative biopsy of the right lobe of the liver is carried out and lymph nodes excised from the lumbar, para-aortic, and mesenteric areas. Stage II patients whose primary lesion is anatomically situated such that deep groin dissection would not be appropriate are managed as follows during the preoperative period: Scalene lymph node biopsy, bone marrow aspiration, and peritoneoscopy with needle biopsy of the liver is carried out. Diagnostic work-up of Stage II patients with an unknown chest lesion culminates in thoracotomy when necessary, to evaluate possible chest metastases. In clinical Stage I patients, found to have positive regional nodes on frozen section during the course of a regional node dissection, laparotomy is carried out under the same anesthetic. If superficial inguinal node involvement is first detected postoperatively, only after studying permanent sections of tissue, then staging laparotomy and deep groin dissection is performed when the patient's clinical condition warrants. During this interval, bone marrow study and scalene node biopsy is carried out as indicated above.

Major Findings:

So far, in approximately 20 patients, three patients were found to have visceral disease which could be detected only by staging laparotomy in two cases and peritoneoscopy in one case. The more accurate assessment of the anatomical extent of the disease has allowed these patients to have more directed application of therapeutic measures.

Significance to Biomedical Research and Program of the Institute:

It is probable that surgical staging will show that more patients have disseminated disease than had been suspected. Such findings, of course, have implications regarding the management of these patients. Patients found to have disseminated disease are not subjected to extensive surgery for control of the primary lesion or the first set of regional lymph nodes. These patients are designated Stage III-S (Stage III-surgically staged), and are candidates for chemotherapy with methyl CCNU or immunotherapy with BCG and allogeneic melanoma cells, in accordance with another National Cancer Institute protocol. Since these patients carry a smaller tumor burden than most Stage III patients, they may have an increased chance of responding to chemotherapy or immunotherapy. Accurate staging of patients and removal from Stage II classification of patients who have occult disseminated disease will eliminate those patients from the Stage II statistical categories and, therefore, allow the effectiveness of surgical procedures and therapeutic lymph node dissections and adjuvant therapies to be better evaluated. The immediate effect of this increased precision in staging will be to show increased effectiveness for the Stage II treatment groups.

Proposed Course:

We will continue to carry out surgical staging of melanoma patients when appropriate. After 25 patients are studied, the data will be analyzed. It is possible that peritoneoscopy might be substituted for exploratory laparotomy in an increased number of these patients. Mediastinoscopy might be substituted for scalene node biopsy, especially in patients with suspicious pulmonary nodules. The effect of the increased precision in staging will be used to reevaluate previous survival statistics. The Stage III-S patients with minimal disseminated disease will be studied in ongoing adjuvant programs for Stage III patients.

Project Description:

Part II. The Surgical Treatment of Melanoma

B. Immunotherapy and Chemotherapy as Adjuvants to Surgical Treatment

Objectives:

To determine the role of adjuvant chemo-immunotherapy in patients with melanoma and breast cancer with small tumor burdens.

Methods Employed:

The Surgery Branch is collaborating with the Immunology Branch and the Chemotherapy Branch on a protocol study to assess the effectiveness of immunotherapy or chemotherapy in patients with minimal residual melanoma. Patients undergo removal of the primary lesions and of the draining regional lymph nodes. If the regional lymph node histological examination confirms the clinical impression of involvement, the patient is then randomized to receive either additional chemotherapy, or immunotherapy, or observation. The chemotherapy arm consists of administration of methyl CCNU by the Chemotherapy Branch. The Immunology Branch administers BCG and allogeneic cultured melanoma cells to the patients randomized to immunotherapy. The observation arm provides an indication of whether chemotherapy or immunotherapy enhances survival and decreases recurrence rate, compared to surgery alone.

The possible utilization of "combination immunotherapy" is being investigated based on experiments performed in mice, guinea pigs, monkeys, and humans which indicate that agents that promote a delayed hypersensitivity reaction increase the responsiveness of circulating lymphocytes to blastogenesis by phytohemagglutinin.

Collaborative efforts with the Medical Breast Service are under way in which the Surgery Branch provides immunological monitoring of patients receiving chemotherapy. The Surgery Branch will also assume responsibility for administration of immunotherapy to patients who will receive this modality in combination with chemotherapy.

Major Findings:

The preliminary status of the Stage II melanoma immuno-chemotherapy protocol prevents conclusions. At the present time ten patients are entered into this study. The phase I study which was carried out in Stage III melanoma patients indicated no prohibitive side effects from the administration of BCG and cultured allogeneic melanoma cells.

Significance to Biomedical Research and Program of the Institute:

The combination of surgery, chemotherapy, and immunotherapy may represent an advance over the use of any of these modalities alone. A combination of agents which promote a delayed hypersensitivity reaction seems to be more potent than single agents in causing enhanced lymphocyte reactivity. It is possible that "combination immunotherapy" like "combination chemotherapy" may be more potent than "single immunotherapy" or "single chemotherapy".

Proposed Course:

The continuing program in these areas will require the cooperative efforts of several groups of laboratories as well as long-term follow-up information. It is proposed to complete these studies until it is determinable whether a combination of agents and what combination of agents is best in these patients.

Project Description:

Part II. The Surgical Treatment of Melanoma

C. Malignant Melanoma: Screening of Potential Surgical Candidates with ^{67}Gallium Scintography

Objectives:

To determine the accuracy and usefulness of the ^{67}Gallium scintigram in the detection of known and unknown metastatic disease in potential candidates for curative surgical treatment of biopsy proven melanoma.

Methods Employed:

Twenty-four sites in each of 48 Gallium scans on 44 consecutive patients with malignant melanoma (a total of 1152 sites) have been evaluated independently by two separate investigators without prior clinical information about the patient. Findings were compared with the results of extensive clinical diagnostic studies performed uniformly on all patients and wherever possible correlated with definitive histologic data. In several patients Gallium activity was assayed directly in selected samples of tumor and adjacent normal tissue obtained at surgery. Autoradiography was performed on these specimens as well.

Major Findings:

Of those sites for which histologic confirmation was available, almost all of the areas positive on Gallium scintigram were subsequently shown to contain malignant melanoma. The overall false positive rate was less than 5%. Those rare instances of false positive Gallium activity were associated with active inflammation or other local abnormality at the site. On the other hand, the overall false negative rate was 19%. This group of false negative scintigram sites accounted for the non-detection of 46% of the total number of melanoma containing sites (only 25% of those lesions greater than 2 cm in size, 73% of those lesions equal to or less than 2 cm). Autoradiography and direct tissue sampling studies confirmed the high affinity of melanoma tissue to ^{67}Gallium with tumor:background tissue ratios varying from 3:1 to 51:1.

Significance to Biomedical Research and Program of the Institute:

The major factor in the relatively low cure rates for surgical treatment of malignant melanoma can be attributed to our inability to recognize clinically silent distant metastases. In this study ^{67}Gallium scintography was shown to be a useful tool to detect those patients with clinically silent metastatic disease prior to surgery. Although some lesions were missed by this technique, the presence of an area of Gallium uptake on the scintigram in an otherwise normal site was shown to be almost diagnostic of malignant melanoma at that site. The ability to detect such metastatic disease will not only provide the basis for avoiding unnecessary radical surgery in incurable patients, but also aid in accurate clinical staging to be used as a guide to the course of palliative immuno-, chemo-, and radiotherapy.

Proposed Course:

The ^{67}Gallium scintigram is now being used routinely to evaluate all surgical candidates for malignant melanoma. Long-term follow-up studies are presently under way on the group of patients studied so far in order to determine whether long-term follow-up will affect the above statistics. Already three of the rare false positives have turned out to have occult disease rendering them now true positives. The use of the ^{67}Gallium scintigram to follow post-operative patients for the appearance of metastatic disease, and to follow Stage III patients in order to assay the progress of immuno-, chemo-, or radiotherapy is currently being evaluated.

Project Description:

Part III. Fine Needle Aspiration Biopsy in the Diagnosis of Solid Tumors

Objectives:

To evaluate the accuracy of results obtained by fine needle aspiration of solid tumors in the diagnosis of malignancy; to evaluate the safety of fine needle aspiration. For over 15 years, Swedish investigators have used fine needle aspiration biopsy techniques to successfully diagnose tumors in breast, salivary glands, thyroid, lymph nodes, prostate, pelvis, and lung. Widespread acceptance of this technique has never been obtained in the United States. Studies carried out to date at NIH have indicated that the procedure is of definite value and can avoid open surgical incisional biopsy in many patients with solid tumors. If it can be demonstrated that the method is highly accurate and that interpretation is satisfactory using Papanicolaou's stain technique, it is possible that the procedure may gain favor in this country and lead to earlier diagnosis of solid tumors as well as assist in the management of metastatic disease.

Methods Employed:

A 22 gauge needle will be used to aspirate undiagnosed solid tumors. A special apparatus developed at the Surgery Branch will permit use of disposable syringes to facilitate use of the technique. Material obtained by aspiration will be smeared on microscopic slides and stained by the Papanicolaou technique. The syringe and needle is then rinsed with saline and this material put through a millipore filter and stained with the Papanicolaou stain. The slides are read by cytologists at NIH and are available for training of cytologists at NIH and other institutions in the United States in the interpretation of aspiration biopsy smears.

Major Findings:

Aspiration biopsy has been carried out on over 250 solid tumors. Diagnoses established by cytological examination have correlated well with diagnoses made upon histologic examination of excised tissue. Accuracy has been 95% in diagnosing tumor in lymph nodes and 93% in diagnosing tumor in subcutaneous nodules. False positive cytologic reports have not occurred. False negative reports occur in about 10% of aspirations. It is anticipated that this will be reduced as familiarity with aspirating technique is gained. In addition, establishment of an aspiration clinic is planned so that prompt examination of the aspirate can be carried out with re-aspiration if necessary. Cells aspirated from tumors will be placed in tissue culture for possible use in future immunotherapy programs and for use in cytological grading of tumors. Fine needle aspiration technique will be used as part of the newly established breast cancer control program at NIH. Aspiration of breast masses will help identify those patients, approximately 90%, whose breast lumps are benign and in whom outpatient excision under local anesthesia can be carried out. Thus unnecessary trauma and expense to the patient can be avoided. As the cytologists have been presented with increasing amounts of material, interpretations of the Papanicolaou smears have become increasingly accurate. Intraoperative aspiration of nodules encountered during surgical dissection in two patients has enabled more accurate planning of the extent of the operative procedure by the surgeon.

Significance to Biomedical Research and Program of the Institute:

Earlier diagnosis of tumors is felt essential for application of therapeutic measures and improved therapeutic results. It is anticipated that fine needle aspiration may provide a fast, accurate method of cancer diagnosis in over 90% of tumor masses aspirated. The avoidance of surgical incisional or excisional biopsy should accelerate establishment of diagnosis and treatment of the patient. Cost should also be significantly reduced. The technique may be applicable to screening clinics and may represent a significant advance in the early diagnosis of cancer.

Proposed Course:

Aspiration with large numbers of solid tumors in various anatomical locations is being carried out. Efforts will be made to expand the use of the technique to physicians throughout the National Cancer Institute. In conjunction with the Cytopathology Department, plans are under way to give instructional courses to cytologists outside the NIH on interpretation of the smears.

Project Description:

Part IV. Evaluation of Uretero-Ileo-Cutaneous Anastomosis Following Radical Surgery for Pelvic Cancer

Objectives:

(1) To provide a method of urinary diversion post-radical pelvic surgery for cancer of cervix, bladder, and certain malignancies of the prostate gland. (2) Compare sigmoid conduit with and without antireflux procedure with

uretero-ileo-cutaneous anastomosis in order to find the better means of urinary diversion to protect the kidneys from the urinary reflux and infection.

Methods Employed:

All patients with biopsy proven cancer of the cervix with radiation recurrence, Stage B_1, B_2 cancer of the bladder, and rhabdomyosarcoma of the prostate have had uretero-ileo-cutaneous anastomosis performed as the method of urinary diversion. Standard surgical and anesthetic procedures have been used for uretero-ileo-cutaneous anastomosis performed on over 250 patients with various pelvic malignancies. Complete electrolytes, serum creatinine, urea nitrogen creatinine clearance, urine culture, intravenous pyelogram, and radioisotope renogram have been performed pre- and postoperatively in following these.

Major Findings:

In over 250 patients undergoing major pelvic surgery for cancer, uretero-ileo-cutaneous anastomosis has been performed as the primary means of urinary diversion. The incidence of calculus formation has been 4.7%, and higher incidence of urinary infection and pyelonephritis. Our data indicate a short segment chosen for conduit would empty better and has less chance of urinary stagnation with residual urine, and consequent urinary tract infection and calculus formation. Also, a high uretero-ileo-cutaneous anastomosis in certain patients is desirable in order to leave the segment of the conduit away from the field of irradiation. However, as far as renal function and electrolytes are concerned, uretero-ileo-cutaneous anastomosis has been superior to the other forms of urinary diversion, especially ureterosigmoidostomy. Radioisotope renograms have demonstrated the backflow reflux of infected urine of the loop into the kidneys has been a problem that needs elimination. Since antireflux operation is not technically feasible with small intestine, we have developed a sigmoid conduit with antireflux operation in experimental animals with excellent results and are utilizing the procedure more often as the technique of choice for urinary diversion in pelvic surgery for malignancies.

Significance to Biomedical Research and Program of the Institute:

A sure, safe, and effective means of urinary diversion is the goal of this search. The renal function, urinary tract infection and reflux with consequent 4.7% calculus formation indicate the urgent need for an antireflux operation in devising a urinary conduit. As the result of these studies we are performing some sigmoid conduit with antireflux procedures to evaluate the long run follow-up and complications in terms of renal function, infection, and calculus formation.

Proposed Course:

Continued operative studies and comparing uretero-ileo-cutaneous anastomosis with antireflux sigmoid conduits.

Project Description:

Part V. Extended Deltopectoral Flap Reconstruction of Anterior Oral Defects

Objectives:

To develop a direct, easily accomplished, and reliable means of reconstruction of defects of the chin, anterior floor of mouth, and tongue that offers a superior cosmetic and functional result to that achieved with conventional techniques of reconstruction.

Methods Employed:

To date, no satisfactory means of reconstructing defects in the chin, anterior floor of mouth, and tongue has been devised. The cosmetic deformity resulting from resection of massive defects in this region and the functional disability, e.g., continuous drool, impairment of speech, prevention of mastication, and pulmonary aspiration of saliva led to an assessment of this heretofore unsolved problem. A flap having its base in the sternal region and extending to a tip in the posterior axillary fold was developed for use in the reconstruction of these defects. The flap has been perfected so it can be raised and rotated without loss of viability of the tip and with minimum inconvenience to the patient.

Major Findings:

The extended deltopectoral flap has been utilized in 21 patients thus far, including 6 who had virtually complete loss of the free tongue. It has allowed complete restoration of speech and swallowing in each case. The technique represents the first cosmetically and functionally satisfactory means of restoration of tissue in such defects.

Significance to Biomedical Research and Program of the Institute:

Impairment of function and the distortion of the appearance of patients having massive resections of tumors involving the chin and anterior tongue region has constantly presented a challenge to the members of the Surgery Branch as well as head and neck surgeons elsewhere. The use of this technique of reconstruction will markedly shorten the hospitalization time of these patients following removal of such malignancies, and allow a rapid and more complete return to their community with elimination of the nursing care required heretofore after such operations.

Proposed Course:

Improvements in the technique of reconstruction utilizing this flap will be made so as to allow its use both at the time of resection of the primary tumor and in those patients who had resection of the tumor at sometime in the past and have residual functional and cosmetic deficits. In addition, this flap has been used in combination with the extended shoulder flap in several patients who had full thickness losses of the face that would have been virtually impossible to construct with currently available techniques. Further improvements in the technique of reconstruction with such flaps should relieve the reticence of the surgeon to resect massive malignancies of the head and neck region which, though technically feasible, would create distortions of appearance and function that would deter the patient from accepting the operation.

Project Description:

Part VI. A Study of the Results of Surgical Treatment and Effects of Adjunctive Therapy in Cancer of the Head and Neck

Objectives:

1. To determine the feasibility of an aggressive surgical approach to irradiation or surgical recurrent malignancies of the head and neck areas.
2. An evaluation of adjunctive chemotherapy used before surgery and its long-term intermittent use after surgery.
3. To determine the effectiveness of a combined intracranial facial approach to cancer of the paranasal sinuses.
4. To determine the prevalence, site of origin, and rationale of treatment of unknown primary disease metastatic to head and neck areas.
5. Evaluation of new methods of surgical approach and reconstruction in surgical treatment of head and neck cancer.
6. The clinicopathological correlation of epidermoid carcinoma of the head and neck area.

Methods Employed:

Following extensive preoperative clinical, pathological, and roentgenological studies, standard and new radical surgical procedures are carried out.

(a) In patients with Stage II, III, and IV epidermoid cancers of the oral cavity treatment with methotrexate is used for 2 to 3 weeks before surgery, and after surgery will be used on a long-term intermittent basis. Surgery will involve wide resection of the primary tumor with unilateral or bilateral neck dissection. An effort will be made to increase survival and decrease the incidence of local recurrence with this combined chemotherapy and surgery approach.

(b) In patients having paranasal sinus cancer, either primary or recurrent, which suggests roentgenographically or clinically that the cribriform plate will be removed at the time of surgery, our approach will be from an intracranial and combined facial surgical incision which will permit en bloc removal of the tumor and at the same time afford protection of the surrounding vital structures.

(c) In patients requiring surgery to control recurrent disease following previous therapy, treatment will be combined pre- and postoperatively with a chemotherapy approach. During our continuing studies for inpatients with head and neck cancer some 70-80% of the patients screened for admission have been failures with previous therapy, usually irradiation. The actual incidence of patients admitted and treated who have been previous failures is 71%. By using chemotherapy before and after surgery it is anticipated that an improvement in survival results will be realized.

(d) In patients requiring large resection for treatment of their cancer immediate plastic reconstruction will be carried out using flaps of tissue from the pectoral or shoulder areas. These flaps will be used singly or in combination at the time of the resection.

Major Findings:

Over the past 3 years, 41 patients have been entered on a pilot combined methotrexate and surgery protocol. No complications have been encountered. The majority of the patients have demonstrated a marked reduction in the size of their tumor and metastatic neck nodes during the 2 week presurgery treatment period. These encouraging results have led to the formulation of a prospective comparison of adjunctive methotrexate and radiotherapy in patients receiving surgical treatment of head and neck cancer.

The combined intracranial facial operation for cancer of the paranasal sinuses has been performed in 53 patients to date. A review of the results revealed that of the patients treated, 19 were dead and 24 were living for a time interval of 4 months to 11 years follow-up. The operation has been extended to include patients showing some involvement of the cerebral meninges. All of the patients who have thus far been approached in this manner have had extensive disease and have not been amenable to treatment in any other fashion. Any survival at all is indeed promising, but more patients and more time is necessary before the procedure can be recommended for routine use. The advantages of the combined procedure are that it involves (1) evaluation of intracranial disease, (2) protection of the brain, (3) avoidance of spinal fluid fistulas, (4) more adequate hemostasis, (5) facilitation of resection, and (6) en bloc resection.

Unknown primary lesions with neck metastases is not a frequent occurrence and accumulation of a series of significant numbers is difficult. The eventual discovery of the primary lesion has usually been in the nasopharyngeal sinus area, but 3 cases have been from the thyroid and 1 from the kidney. In the past year, 6 new cases have been admitted to this study.

Significance to Biomedical Research and Program of the Institute:

These locally resistant, usually recurrent head and neck lesions have long been a problem to oncologists. We are attempting to correlate aggressive surgical exploration of these lesions with survival and the mode of tumor dissemination. Careful environmental background reviews reflect the close relationship between the Surgery Branch and the Dental Prosthetic Unit allowing a comprehensive evaluation of the indications for plastic reconstruction of facial deformities and evaluation of newer prosthetic materials.

Proposed Course:

The extent of surgical excision cannot be much increased in its scope beyond that which we are now able to perform successfully. More patients are necessary before the combined craniofacial approach to paranasal sinus cancer can be recommended for routine use. Both procedures are based on sound experimental and clinical observations and final evaluation should prove rewarding. Pilot studies are under way to determine the feasibility of intensive preoperative medication in patients with disease which has locally extended beyond that which can be cured by conventional means.

Project Description:

Part VII. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

Objectives:

A review of patients operated on by the Surgery Branch of the National Cancer Institute for sarcomas of bone as well as soft tissue sarcomas primarily involving the trunk and extremities was undertaken. The questions of survival statistics, incidence of regional node metastases, and the incidence of local recurrence following surgery were of particular interest. Also of interest were the results of therapy at the National Cancer Institute as compared to the published results country-wide.

Methods Employed:

Case records of patients operated on by the Surgery Branch from 1953 until December 31, 1968, were reviewed. Patients studied were those who underwent definitive surgery at this institution.

Major Findings:

Sixty-two patient records were reviewed in detail. The survival statistics for 2 years and greater demonstrated the following: rhabdomyosarcomas, 3 of 8; liposarcoma, 2 of 4; undifferentiated sarcomas, 1 of 4; fibrosarcomas, 7 of 14; chondrosarcomas, 8 of 11; synovial sarcomas, 1 of 1; mesenchymal sarcomas, 1 of 2; osteogenic sarcomas, 2 of 16 (1 of these 2 was a parosteal sarcoma); leiomyosarcoma, 1 of 1.

This gave an overall survival rate of 26 out of 62 patients.

In this 15 year period there were 21 hemipelvectomies performed and 9 interscapular thoracic amputations performed. The mortality from this extensive surgery was minimal, only 1 patient of these 62 died in the postoperative period and that was a patient who died of renal failure after a combined total pelvic exenteration-hemipelvectomy.

There were 4 local recurrences, 1 following radical local excision, 2 following interscapulothoracic amputation, and 1 following hemipelvectomy. These local recurrences were all in the patients who had massive local lesions.

Significance to Biomedical Research and Program of the Institute:

Survival statistics and local recurrence rates were comparable to published reports. Of particular importance was documentation of the extremely poor outlook for the patient with an osteogenic sarcoma, with the survival being 10% or less at this institution. The other common lesion treated here with a poor prognosis was fibrosarcoma, with a 50% survival rate greater than 2 years. These data have suggested that we can select patients on the basis of their histological diagnosis and the size of the lesion to represent very poor risk patients and include them in some type of an adjuvant protocol. At present only the patients with metastatic cancer have been treated by immunotherapy, but certainly patients with an expected survival or only 10% would be an excellent group to attack aggressively with immunotherapy. Since osteogenic sarcomas presumably do contain a common tumor-specific transplantation antigen, aggressive immunotherapy seems indicated. In addition, patients with osteogenic sarcomas or large soft tissue sarcomas would be acceptable for aggressive adjuvant chemotherapeutic protocol.

Proposed Course:

The patients who have been operated on since January 1, 1969, will be reviewed to keep the study up to date. This data is being made available to the National Sarcoma Task Force for country-wide evaluation of patients with soft tissue sarcomas. Further evaluation of rehabilitation of patients who have been amputated is being undertaken to better evaluate patients preoperatively and to assist them in postoperative convalescence. The data is being looked at closely from the standpoint of seeing with which tumors we can avoid radical amputative surgery or particularly in patients whose prognosis is grim.

Project Description:

Part VII. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

A. Hemipelvectomy: Its Role in the Treatment of Extremity Sarcoma

Objectives:

To develop a technique of hemipelvectomy that minimize the skill required, the operative time, and blood loss entailed.

Methods Employed:

As a result of the reluctance of surgeons to perform hemipelvectomy when indicated, because of the technical skill required in the execution of the operation, lesser procedures which lead to a higher recurrence rate and mortality rate are being employed on a widespread basis. In an effort to improve the survival rate with such patients, we have recently completed a study of the operation in cadavers and living patients and devised a technique that is within the skills of the average general and orthopedic surgeon.

Major Findings:

The conclusion of an assessment of the problem was that the most difficult aspect of hemipelvectomy as conventionally performed was the deep pelvic dissection. We then found that the relatively limited exposure to this anatomically complex and vascular area afforded when the operation is performed with the patient in the supine position is obviated with the patient in the lateral decubitus position. The addition of the Trendelenburg position then afforded additional exposure because of the gravitational shift of the viscera into the upper abdomen. In addition, simulnaneous access to the anterior and posterior aspects of the pelvis resulting from this patient position, as well as the perineum when the extremity was elevated, allowed the rapid execution of the operation, particularly in those instances in which uncontrollable bleeding was encountered during the deep pelvic dissection. After studies in cadavers suggested that these concepts should be assessed in living patients, the operation was so utilized with such great success that it was adopted as the standard technique by the Surgery Branch.

Significance to Biomedical Research and Program of the Institute:

The alarming number of lower extremity malignancies referred to this Institute after recurrence with inadequate operation because of the reluctance of the referring physician to perform hemipelvectomy indicates the need for an easily mastered technique for the operation. The technique developed should have a favorable effect on the surgeon's willingness to perform this operation as the initial procedure, thus giving the patient the maximum opportunity for cure. In addition, the utilization of this technique by the Surgery Branch has shortened the operative time and the blood loss entailed in the operation, and allowed the safer performance of these operations in relatively poor risk patients.

Proposed Course:

A detailed and illustrated disclosure of the technique for publication in a surgical periodical is in progress. In addition, a motion picture depicting the advantages of the operation as well as a detailed step by step conduct of the operation in a patient has been completed and submitted to the Film Library of the American College of Surgeons to make it available for distribution to general and orthopedic surgeons.

Project Description:

Part VII. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

B. The Use of Coumadin Anticoagulation as an Adjunct to the Surgical Treatment of Sarcomas

Objectives:

Patients with extremity sarcomas thought to be curable by surgery have a poor survival, approximately 20% at five years. Perioperative anticoagulation in animal tumor systems has demonstrated a marked increase in survival or a decrease in incidence of pulmonary metastases. Warfarin anticoagulation is to be tested in the clinical setting to see if it will improve the survival of patients with extremity sarcomas amenable to surgical amputation.

Methods Employed:

Patients with histologically proven sarcoma thought to be curable by amputation will be admitted to the study. Evidence of metastatic disease or any contraindications to anticoagulation will exclude the patient from the study. The patients are begun on oral warfarin one week prior to surgery. Anticoagulation is to be monitored by the standard prothrombin time, prothrombin-proconvertin levels, and specific assay of plasma factors VII-X, VIII, and IX. The prothrombin time is to be maintained 6-7 seconds above control (10.0 - 11.5 second range). The P&P test is to be maintained in a range of 12.5 - 25%. Drugs known to interfere with the metabolism or uptake of warfarin are to be avoided.

Major Findings:

Twenty patients have been included in the protocol to date. Twelve of 20 patients are alive and free of metastases at this time. There have been 14 osteogenic sarcomas operated on, 7 of which are alive at periods of 3 months, 8 months, 11 months, 15 months, 2-1/2 years, and 4 years. Six other patients with various histologic types of sarcomas have been anticoagulated, 5 of which are alive at periods of 5 months, 7 months, 23 months, 2-1/2 years, and 3 years. Six major wound problems have been attributed to anticoagulation. Bleeding complications were encountered in 8 patients necessitating stopping the warfarin anticoagulation for at least a short period of time. All of these patients were over anticoagulated according to our criteria.

Significance to Biomedical Research and Program of the Institute:

A very high mortality rate continues to occur in patients with sarcoma, despite radical amputative surgery. For this reason, effective adjunctive therapy must be searched for in this disease. It is hoped that anticoagulation will improve the results of surgical treatment for sarcoma patients. A greater understanding of the mechanism involved in tumor metastases is also sought.

Proposed Course:

Patients will be randomized to either control or warfarin therapy instead of being compared with historical controls. The level of anticoagulation will be followed much more closely with the assistance of the Hematology Section of the National Cancer Institute. It is felt that closer monitoring can reduce the hemorrhagic complications that have been encountered.

Project Description:

Part VII. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

C. Medullary Extension of Osteosarcoma

Objectives:

To determine the extent of clinically unappreciated intramedullary extension of osteosarcoma in clinical cases.

Methods Employed:

Amputation specimens from the clinical population of the Surgery Branch, NCI, are examined in cooperation with the Laboratory of Pathology. Radiographic (preoperative) and gross pathologic characteristics were correlated. Microscopic examination of specimens is performed.

Major Findings:

Radiologic and gross pathologic extent of tumor have been found to be similar in this series. Unappreciated medullary extension of osteosarcoma was absent. Dissemination of foci of osteosarcoma within the medullary cavity, remote from the main tumor mass, separated by so-called "skip areas", have not been found.

Significance to Biomedical Research and Program of the Institute:

Varying investigators have reported conflicting results as to survival when osteosarcoma is treated by amputation. Many centers report equal survival rates with transmedullary (conservative) amputation to those obtained by radical amputation. The advocates of radical amputation maintain that isolated foci of tumor, beyond uninvolved "skip areas" in the medullary canal, are often present and are left following conservative amputation. A systematic pathologic study of clinical specimens could help to elucidate whether the radical approach, with its increased morbidity, has a scientific basis.

The results of this study, and one other study in the literature, suggest that there is no pathologic basis for advocating radical amputation in osteosarcoma. Coupled with similar survival rates, these findings support the view that transmedullary amputation, with its vastly lessened morbidity, may by the procedure of choice for selected osteosarcomas.

Proposed Course:

Continued comparison of the microscopic extent of tumor found in excised bones and comparison with the extent determined radiographically will be pursued for determination of the feasibility of a study of the role of transmedullary amputation in patients with osteosarcoma in the distal portion of long bones.

Project Description:

Part VIII. An Improved Technique for Radical Groin Dissection

Objectives:

Standard techniques of radical groin dissection entail significant immediate and long-term morbidity. The immediate morbidity is primarily due to necrosis of the skin flaps leading to infection of the operative wound, thrombosis of the femoral vessels, and thromboembolism. Prolonged hospital stays occur in approximately 1/2 of patients having this operation. The long-term morbidity consists of persistent and incapacitating lower extremity edema with limitation of motion and skin ulceration. This study was undertaken to develop an improved technique of radical groin dissection which would obviate these morbidities.

Methods Employed:

Through a trial of all existing techniques and study of the anatomical factors involved in radical groin dissection, a technique was devised that utilizes two incisions. An oblique incision paralleling the inguinal crease is used to dissect the superficial inguinal lymph nodes. A second, midline incision is used to dissect the pelvic lymph nodes. In this technique, the circumflex iliac and inferior epigastric vessels are preserved.

Major Findings:

Using the technique described, thus far 52 patients have had radical groin dissections. Twenty-seven of these were unilateral and 25 were bilateral radical groin dissections. The immediate morbidity due to flap necrosis has been minimal, and in no case has delayed the ambulation of the patient. The long-term result has likewise been gratifying. None of these patients has developed extremity edema or morbid skin changes.

Significance to Biomedical Research and Program of the Institute:

An important contribution to the treatment of cancer patients would be the reduction of the morbidity associated with radical groin dissection. This operative technique appears to have achieved this goal. This is reflected in decreased postoperative hospitalization time and concomitant or rapid return to preoperative functional capacity of the patients who had radical groin dissection by this operative approach.

Proposed Course:

Continued study of this technique is in progress with an attempt to further improve it and adopt it for all situations requiring radical groin dissection.

Project Description:

Part IX. The Role of Bacterial Infection in Cancer Surgery

Objectives:

To further substantiate findings in the previous years of the evaluation of the operative wound infection rate and its relation to the "prophylactic" use of antibiotics following extensive cancer surgery.

Methods Employed:

In the past year the immediate admission and repeated preoperative culturing of all patients' nose, skin, and tumor allowed patients to be classified into either carrier or non-carrier status of hemolytic staphylococcus aureus coagulase-positive organisms. By bacteriophage typing, through the cooperation of the Clinical Pathology Department, a correlation could be drawn between the patient's preoperatively carried organism and that carried postoperatively, either asymptomatically or related to a postoperative infection. In previously reported studies patients undergoing radical cancer surgery had, on a double blind basis, either 10 days of chloromycetin postoperatively or a placebo. A subsequent study entailed the use of 7 days of chloromycetin postoperatively in all patients, while on a double blind basis the patients received preoperatively either 3 days of placebo or 3 days of antibiotic. The current protocol utilized intravenous antibiotics before, during, and 7 days following the operation. Following 2 separate studies, which have been reported, antibiotic therapy has been used prophylactically in a therapeutic manner on patients undergoing cancer therapy. These patients have been carefully observed for wound infection and any possible correlation to their "carrier status" of pathogenic organisms.

Major Findings:

The previous annual reports detail the specific results of both double blind antibiotic studies. In brief, the infectious complication rate following extensive cancer surgery was lowered from 54% to 14% by the use of pre- and postoperative antibiotics rather than placebo. An 8% infection rate occurred when the intraoperative use of antibiotics was employed in addition to pre- and postoperative coverage.

The overall results for the last 18 years can be seen in the accompanying table. The 1960 rate was elevated to 32% in that this was the year of the original study, whereas 1959 was lowered somewhat because of the discontinuance of the use of penicillin and streptomycin and the selection of antibiotics was left to the discretion of the surgeon.

18 YEAR SUMMARY OF INFECTION RATES AND STAPHYLOCOCCAL INCIDENCE FOLLOWING EXTENSIVE CANCER SURGERY

Year	Extensive cancer operations	Infection rate percent	Per cent of total infections that were staphylococcal
1956....	128	28	35
1957....	139	31	42
1958....	145	30	59
1959....	130	22	69
1960....	144	32	74
1961....	115	13	73
1962....	132	10	72
1963....	137	15	70
1964....	131	7	51
1965....	132	6	36
1966....	141	5	22
1967....	128	6	17
1968....	209	8	14
1969....	217	10	8
1970....	132	11	7
1971....	197	13	4
1972....	208	19	2
1973....	210	17	5

During the past 18 years, 2775 extensive cancer operations have been performed by the Surgery Branch of the National Cancer Institute. During the early part of this study the infectious complications ranged from 22% to 31% during a time when penicillin and streptomycin were used somewhat routinely at the discretion of the surgeon. The results of a double blind antibiotic-placebo study revealed an infection rate of 54% when a placebo was used and 14% when routine 10 day postoperative antibiotic therapy was instituted. A subsequent double blind study showed that by giving drug for 3 days preoperatively and 7 days postoperatively antibiotic therapy resulted in a 17% rate of infectious complications. With continued use of antibiotic prophylaxis, while continuing the same type of extended operative procedures, the infection rate was 10% and 15%. Chloramphenicol, 2 gm every 6 hours, was the antibiotic used and there were no significant complications referable to drug toxicity and only 5 severe infectious complications referable to loss of staphylococcal sensitivity to the drug.

Over the past years the percentage of total infections that were hemolytic staphylococcus aureus DNA-ase positive in origin progressed from 35% in 1956 to 70-75% in 1961-64, and dropped to 36% in 1965 and 14% in 1968, and was 50% in 1973. At the same time, the incidence of patients who were carriers of staphylococci on admission to the hospital was 22% and has remained at that approximate level. In the past, patients who carried staphylococci into the operating room, usually asymptomatically, ran at 38% increased rate of infection over non-carriers. Ninety-three percent of these patients who subsequently become infected as an operative complication will become infected with the same phage type organism that they carried into the operating room. This suggests that self-infection may play as important a role in infectious complications as does cross-contamination. Of greater importance at this time is the alarming increase in the complications associated with Pseudomonas and Klebsiella and E. Coli infections. So frequently death is the result of a septicemia from these organisms. They are much more of a problem today both in prevalence and in morbidity and mortality than the staphylococcus. The gradual increase in wound infections which we are again noting, from a low of 5% in 1966 and now 25% in 1973, is in part due to newly emergent organisms which have in the past been uncommon as a threat. However, the chief reason which has not yet been demonstrated by a controlled study is apparently related to the lack of uniformity in prophylaxis in the administration of prophylactic antibiotics for patients undergoing extensive cancer procedures. Justified trend in the conservative use of antibiotics in patients undergoing general surgical procedures is seemingly leading to increased morbidity in these cancer patients. In addition, the trend on the part of the resident staff, who are from general surgical residencies without significant cancer orientation, is to use antibiotics rather sparingly. This is a procedure well documented in general surgical cases in contrast to our experience citing the need for "prophylactic" antibiotics in cancer patients.

Significance to Biomedical Research and Program of the Institute:

When postoperative morbidity and mortality are increased due to complications related not directly to the cancer but due to infection, the overall evaluation of surgery and its place in cancer therapy is markedly altered. Bringing an alarming infection rate under relative control has decreased morbidity and mortality as indicated in the previous paragraphs. In a hospital such as this where rather elaborate facilities are available for close patient surveillance, studies such as this can be done which can be of significant value to the practicing surgeon. The fact that the total infectious complication rate seems to be rising may be related to the reluctance on the part of the clinician to use Chloramphenicol which has been shown over the years to be the drug to which the hospital organisms are most susceptible. Because of the medicolegal aspects related to Chloramphenicol administration, the resident staff use this antibiotic only on direct insistence on the part of the senior staff. The continued decrease in the staphylococcal infection complication rate is consistent with findings in most hospitals throughout the country.

Proposed Course:

The indications for "prophylactic" antibiotic usage when carrying out the present clinical investigative studies within the Surgery Branch are unequivocal. Close surveillance of carrier patients and personal carriers within the hospital is being continued. It is anticipated that continuation of the surveillance program will not only lessen the so-called epidemic infectious outbreaks occasionally seen on all surgical wards but will allow more rapid and adequate control when such does occur. It is proposed that the critical period of antibiotic coverage is at the time of the operative procedure itself and that constant infusion and drug level maintenance of antibiotics during this period is mandatory in cancer surgery.

Project Description:

Part X. Treatment of Breast Cancer

Objectives:

The National Cancer Institute intramural breast cancer program is evolving as a closely integrated endeavor of the Medical Breast Cancer Service and the Breast Oncology Section of the Surgery Branch to develop more effective methods of diagnosis and treatment of all stages of breast cancer ranging from minimal non-palpable lesions to far advanced metastatic disease.

Methods Employed:

Since its inception in September 1971, the Breast Tumor Service has developed as a truly interdisciplinary approach to breast cancer. Admissions to the study programs have come through both the Surgery Branch and Medicine Branch. A further step in the cooperative approach between these two branches has been the development of a Breast Problem Clinic (BPC) which evaluates all new patients including consultations generated within the Clinical Center. Staffed by members of both Branches, the BPC examines patients with benign as well as malignant disease according to protocols which have been developed. It is hoped that a systematic work-up through this specialized clinic will lead to a clearer understanding of problems associated with diagnosis and extent of disease prior to assignment of patients to treatment protocols.

By looking at the earliest manifestations of cancer as currently detected by mammograms and xerograms or as found in opposite breast biopsies, it is hoped that treatment of minimal cancer will be followed by decrease in mortality rates when undertaken on a larger scale. Primary treatment of breast cancer is being undertaken with conventional radical or modified radical mastectomy, combined in poor risk patients having positive axillary nodes with postoperative adjuvant chemotherapy and/or immunotherapy. Combining adequate local treatment with systemic treatment will hopefully improve survival and diminish mortality in those patients with more than three positive nodes.

Patients with negative axillary nodes are being followed during the disease-free interval, and evaluated periodically for evidence of metastatic disease as well as for indication of a new primary cancer in the opposite breast. Investigations in the value of tumor markers and radioisotope scans are included in these studies along with conventional radiologic examinations and thorough physical examinations.

Protocols have been developed to deal with recurrent and disseminated disease in both premenopausal and postmenopausal women, thereby augmenting a comprehensive program which is capable of dealing with all manifestations of breast cancer.

Mindful of the basic problems concerned with cause and prevention, collaborative studies are under way with the Laboratory of Pathology, the Cytopathology Section, the Experimental Biology Projects Section, the Laboratory of Biochemistry, and the Laboratory of Chemical Pharmacology. Other cooperative studies are ongoing in the Tumor Cell Biology Laboratory, Radiation Branch, Department of Nuclear Medicine, Department of Diagnostic Radiology, and the Laboratory of Cell Biology.

The scope of ongoing treatment protocols which require Surgery Branch services including diagnostic work-up, staging, biopsy, definitive primary surgical care, palliative resection, and endocrine ablation procedures are illustrated by the following:

1. BTS 122: Metastatic Breast Cancer Carcinoma Study to Evaluate the Effect of Cyclophosphamide, Methotrexate, 5-Fluorouracil versus Cyclophosphamide, Adriamycin, and 5-Fluorouracil. This study will evaluate eligible patients who have not received previous chemotherapy, but who present with, or are referred with, metastatic breast cancer.
2. BTS 123: Study to Evaluate the Effect of Oophorectomy with and without Adjuvant Chemotherapy in the Management of Recurrent Mammary Carcinoma in Premenopausal Women. Patients entering this protocol will have an oophorectomy and the responders will be randomized to cyclophosphamide, methotrexate, 5-FU, or to no therapy.
3. Protocol B124: Study to Evaluate the Effects of Estrogen Therapy with and without Additive Chemotherapy in the Management of Recurrent Mammary Cancer in Postmenopausal Women.
4. Protocol B131: Evaluation of Adjuvant Chemotherapy in Breast Cancer with no Evidence of Disease Following an Excised Mastectomy Site Recurrence. This protocol will test the hypothesis that local recurrence at the operative site is frequently a manifestation of general dissemination. The impact of adjuvant chemotherapy following excision of the local recurrence upon the time to clinical recurrence and survival will be compared to no chemotherapy until clinical recurrence.

5. Protocol B132: Comparative Trial of Nafoxidine and Fluoxymesterone plus Nafoxidine in Metastatic Breast Cancer. Patients entering this study will be randomized to one of the two treatment arms and will be followed until failure to respond or relapse. There is no crossover or continuation of follow-up under this study.
6. Protocol B133: Phase I-II Evaluation of a Combination of Adriamycin and Dibromodulcitol in Metastatic Breast Cancer.
7. Protocol B134: Phase I-II Evaluation of Dibromodulcitol in Previously Treated Patients with Metastatic Carcinoma of the Breast.
8. Protocol B135: Evaluation of a Combined Modality Approach to Therapy in Mammary Carcinoma Patients with T_c 1, 2, 3a, 4b N+ Lesions. This is the basic protocol for adjuvant chemotherapy of surgically treated patients who have positive axillary nodes. Such patients are currently being randomized between the chemotherapy arm consisting of cyclophosphamide, methotrexate and 5-FU and an observation arm. A third arm consisting of the same chemical combination plus immunotherapy is contemplated.
9. Non-treatment protocols include the following studies currently being undertaken on all patients under the purview of the Breast Tumor Service:
    - (a) Protocol B13A: Evaluation of Tumor Markers in Patients with Breast Cancer.
    - (b) Protocol B13B: Evaluation of Hormone Levels in Patients with Breast Cancer.
    - (c) Protocol B13C: Evaluation of Prognostic Usefulness of Barr Body Evaluations in Breast Cancer.
    - (d) Protocol B13D: Evaluation of Chromosomes in Patients with Breast Cancer.

Significance to Biomedical Research and Program of the Institute:

The aim of the Breast Cancer Service is to further the understanding of breast cancer and to improve survival and diminish mortality rates through a collaborative effort using every available modality for study and treatment.

Proposed Course:

Expansion and further development of currently instituted protocols is planned. Improvement in organizational and administrative procedures within the program is contemplated as a means of facilitating the large array of ongoing studies.

Project Description:

Part XI. Testis Cancer

Objectives:

The goals of this project have been: (1) Study of newer tumor markers in relation to early detection or recurrence and documentation of postsurgical residual cancer. The markers included in this study are placental lactogen oncofetoprotein, placental alkaline phosphatase and chorionic gonadotropin.

(2) The role of retroperitoneal lymph node dissection in relation to the serum and urinary levels of these tumor markers and detection of the survival rate. (3) Cell culture of different testicular cancer in an attempt to measure cellular immunity in vitro and in vivo in relation to the different stage of testis cancer.

Methods Employed:

The urinary and serum tumor markers are measured by different techniques including radioimmunoassay of alphafetoprotein. The in vivo cellular immunity is detected by DNCB and other skin tests. A modified midline surgical technique developed in the Surgery Branch of the National Cancer Institute has been utilized for a total retroperitoneal lymph node dissection. The main advantage of this technique has been complete bilateral removal of the retroperitoneal lymph nodes with preservation of the contralateral sympathetic nerves at the L_1 and L_3 in order to preserve the ejaculation in young males. The specimens have been utilized for cytogenetic, light, and electron microscopic and tissue culture studies.

Major Findings:

The contralateral retroperitoneal lymph nodes have been found to contain cancer cells in 10% of the cases. In 5% of the cases, the contralateral lymph nodes were positive although the ipsilateral retroperitoneal lymph nodes in the vicinity of the renal pedicle were free of tumor. Thus, important histopathologic findings indicate that a bilateral retroperitoneal lymph node dissection should be employed in order to remove all the involved lymph nodes. In 80% of testicular teratocarcinoma and embryonal carcinoma the alphafetoproteins have been elevated and it seems to correlate with the tumor activity and recurrence. We have used this tumor marker for detection of residual tumor and in follow-up of the patient.

Significance to Biomedical Research and Program of the Institute:

The chorionic gonadotropin detected by the conventional technique has been utilized in following testicular cancer, but it has been found to be elevated only in 10% of testicular cancer. Since our findings of elevation of alphafetoprotein in 80% of the patients with testicular cancer, we feel it is more reliable in follow-up of patients with testis cancer. The detection of the reliability of placental alkaline phosphatase and lactogen tumor marker will have a significant contribution in detection of tumor and tumor recurrence. The correlation of the host resistance with the staging of the tumor and its implication in newer therapy such as immunotherapy are being studied.

Proposed Course:

The continuation of the project with accumulation of statistically significant number of cases will be helpful elucidation of the tumor markers, immunobiology, newer surgical and chemotherapeutic procedures in testis tumors. The preliminary studies of the cytogenesis of these tumors have indicated abnormal chromosomes associated with testis tumors. The detection of the exact chromosomal abnormalities is critical in understanding the pathogenesis of these multipotential tumors.

Project Description:

Part XII. Use of ^{111}Indium Labelled Bleomycin in Evaluation of Neoplastic Disease

Objectives:

1. To assess the affinity of isotopically labelled Bleomycin for primary and metastatic neoplasms via total body rectilinear scanning and regional gammagraphic exploration.
2. To exploit this expected affinity in the spheres of diagnosis and management by pursuing serial gammagraphic studies prior to and following completion of adjuvant chemotherapy and/or definitive surgery.

Methods Employed:

Bleomycin labelled with the gamma emitter ^{111}Indium is employed at a dosage of 40-60 microcuries per kilogram. Forty-eight hours following administration of isotope, total body rectilinear scanning is conducted. In cases of tumors with propensity for lymphatic dissemination, gammagraphic camera views of the primary site and regional node bearing areas are obtained. Findings are to be compared with results of routine clinical diagnostic studies performed on all patients admitted to the Surgery Branch.

Major Findings:

Since the inception of the study (February 1974) only eleven patients have been studied. There appeared to be no false positives and no false negative results when compared with clinical findings, roentgenographic findings and other radionuclide studies. As yet, however, sample size is too small to permit accurate statistical analysis of the results.

Significance to Biomedical Research and Program of the Institute:

It is anticipated that Bleomycin scanning will prove to be a useful tool in the detection of clinically inapparent disseminated malignancy, thus permitting more prompt institution of modalities of therapy other than surgery. Furthermore, it is anticipated that serial Bleomycin scanning may aid in effecting earlier diagnosis of clinically occult regional metastasis. This, in turn, might permit more prompt institution of aggressive regional surgical therapy.

Proposed Course:

Bleomycin scanning will be conducted on all patients admitted to the Surgery Branch of the National Cancer Institute. Following the acquisition of a statistically significant sample, data will be critically analyzed and assessed. Should the scan prove to be a useful modality in cancer diagnosis and management, efforts will be made to expand its use.

Publications:

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Geelhoed, G.W., Kane, M., Dale, D., and Wells, S.A.: Colon ulceration and perforation in cyclic neutropenia. J. Ped. Surg. 8:379-382, 1973.

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DeKernion, J.B., Tynberg, Peter, Persky, Lester, and Fegen, J.P.: Carcinoma of the penis. Cancer 32:1256-1262, 1973.

Geelhoed, G.W., Graff, K.S., Duttera, M.J., and Henderson, E.S.: Acute leukemia presenting as a breast mass. J.A.M.A. 223:1488-1489, 1973.

Javadpour, N., Dellon, A.L., Vermess, M., and Klippel, J.H.: Intrarenal arteriovenous fistula. Early surgical repair with preservation of renal parenchyma. Urology 1:457-459, 1973.

Javadpour, N., Dellon, A.L., and Kumpe, D.A.: Multilocular cystic disease in adults. Imitator of renal cell carcinoma. Urology 1:596-599, 1973.

Javadpour, N.: Immunologic features of genitourinary cancer. Urology 2:103-108, 1973.

In Press:

Geelhoed, G.W., and Ketcham, A.S.: Pelvic neoplasms causing pain during coitus. Med. Aspects of Human Sexuality.

Geelhoed, G.W., and Myers, G.H., Jr.: Primary melanoma of the male urethra. J. Urol.

Serial No. NCI-3805
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Studies of Mechanisms of Metastases

Previous Serial Number: Same

Principal Investigator: A.S. Ketcham, M.D.

Other Investigators: P.B. Chretien, M.D., W.F. Sindelar, M.D.
H.C. Hoover, M.D., G.B. Bulkley, M.D. and
Hilda Wexler

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total:	3-1/2
Professional:	1
Other	2-1/2

Project Description:

- Part I. Acetylsalicylic Acid Effect on Platelet Aggregation and Tumor Metastases
- Part II. The Effect of Combined Coumadin and Adriamycin Treatment on Tumor Metastases in Mice
- Part III. Metastases Kinetics and the Role of Host Immunity in the Metastatic Process
- Part IV. The Metastatic Potential of Experimental Pulmonary Metastases
- Part V. Ultrastructural Features of the Formation of Pulmonary Metastases

Project Description:

Part I. Acetylsalicylic Acid Effect on Platelet Aggregation and Tumor Metastases

Objectives:

It has been shown by Gasic, et al., that thrombocytopenia can cause a decreased number of pulmonary metastases in animals receiving an intratumor cells. Preliminary evidence has also shown that platelet aggregation may be of importance in the lodgment and growth of tumor cell emboli in the vascular system. Acetylsalicylic acid (ASA) has been shown to paralyze the platelet's ability to aggregate. There have been some studies showing that ASA can decrease the number of pulmonary metastases in mice. Further work by Dr. Gasic, et al., has pointed out that some tumors have the ability to cause platelets to aggregate, while other murine tumors do not possess this ability. Using these findings, he has been able to predict those animal tumor systems wherein ASA treatment would cause a decrease in number of metastases.

Methods Employed:

Experimental animals were given ASA in doses ranging from 15 mg/kg/24 hours to 250 mg/kg/24 hours, by adding it to their drinking water. Bleeding times were performed by making a uniform cut in the tail and measuring the time that bleeding ceased. The effect of ASA on platelet aggregation was determined by use of a chronolog aggregometer. The ability of different murine tumors (MCA-induced fibrosarcomas) to initiate platelet aggregation were tested. ASA effects on tumor metastases were examined by injecting methylcholanthrene-induced fibrosarcoma cells (approximately $1x10^5$) into the footpad of mice. These tumors were allowed to grow to 1-1/2 to 2 cm in diameter, at which time the tumor-bearing extremity was amputated. All mice were then followed for an additional three weeks, at which time they were sacrificed, the lungs inflated with India ink, and the number of tumor metastases determined macroscopically. Mice treated with ASA were compared to controls receiving tap water.

Major Findings:

We have found that the bleeding time of mice is quite variable, although the animals treated with ASA in dosages of 100 mg/kg/24 hours do have a prolongation in their bleeding time as compared to controls. ASA-treated animals demonstrated a decrease in platelet aggregation when stimulated with 1 mcg of adrenalin. The results of ASA treatment in controlling metastases in one murine tumor system showed 76% incidence of metastases in the control group as compared to 57% in the ASA treated group ($P<.025$). This particular MCA-induced tumor had very weak platelet aggregating ability. However, another experiment using a tumor known to be a strong platelet aggregator failed to show a significant difference with ASA. Further studies are in progress.

Significance to Biomedical Research and Program of the Institute:

If various human tumors are shown to be strong stimulators of platelet aggregation, their metastatic potential may be altered by such agents as ASA.

Proposed Course:

Human tumors of various histologic types will be tested for any propensity to aggregate the host's platelets. A protocol for treating patients with ASA will be developed if warranted by further studies. Multiple animal tumor systems will be studied comparing non-platelet aggregating and platelet aggregating tumors and the effects of ASA on their pattern of metastases.

Project Description:

Part II. The Effect of Combined Coumadin and Adriamycin Treatment on Tumor Metastases in Mice

Objectives:

There is considerable evidence in the literature suggesting that the blood coagulation system has an effect on tumor metastases. Hypercoagulative states have been shown to result in increased numbers of metastases as compared to hypocoagulative states in experimental animals receiving a bolus of tumor cells injected intravenously. There is evidence to show that tumor cells can produce a thromboplastin-like material, that fibrin deposited around tumor cells is related to growth and division of tumor cells, and that a fibrin coat around tumor cells may protect them from the effects of cytotoxic agents. The purpose of this project is to see if the combination of Coumadin anticoagulation and Adriamycin is superior to Adriamycin alone in decreasing pulmonary metastases.

Methods Employed:

Mammary carcinoma cells were injected into the footpad of mice. The tumors were allowed to grow to sufficient size that metastasis to the lungs would occur. The primary tumor was then amputated. After three weeks, the mice were sacrificed and the number of tumor metastases determined macroscopically by differential staining of lung and tumor tissue with an India ink solution. One-half of the mice received tap water throughout the experiment, the other half of the mice received sodium warfarin added to their drinking water. The warfarin was started at the time of tumor cell injection and continued until sacrifice. Prothrombin times were done on mice receiving tap water and those receiving Coumadin in groups of 10 over the period of the experiment. On the second day following amputation, the tap water the Coumadin groups of mice were divided in half, each receiving either saline or Adriamycin in a dose of 7 mg/kg.

Major Findings:

The effects of warfarin anticoagulation alone upon this particular mouse tumor system has been shown to decrease the incidence of pulmonary metastases in these mice from 81% in the control animals receiving tap water to 30% in the animals receiving sodium warfarin. Effects of Adriamycin alone showed significant improvement over controls. Adriamycin and coumadin combined showed the most significant improvement (81% incidence in controls to 21% incidence in treated group - $P<.0005$).

Significance to Biomedical Research and Program of the Institute:

There are several clinical studies suggesting that anticoagulation combined with chemotherapy is superior to chemotherapy alone. Very little data has been accumulated in animal tumor systems to substantiate these effects. It is hoped that further studies in the animal tumor systems may increase our knowledge of the role that the coagulation system plays in the initiation and growth of blood-borne metastases. These same mechanisms may also aid us in treating tumors with chemotherapeutic agents.

Proposed Course:

The methods as outlined are to be applied to several different tumor systems. Tumor metastases induced by intravenous injection of tumor cells will be used and the effects of Adriamycin and anticoagulation will be evaluated.

Project Description:

Part III. Metastases Kinetics and the Role of Host Immunity in the Metastatic Process

Objectives:

It is desirable to quantitate the time course of the appearance of metastases in a uniform population of animals in much the same way that tumor growth has been quantitated. Using this approach, it is then possible to observe the role of developing host immunity in the appearance, and in some cases regression, of metastatic foci.

Methods Employed:

Through careful control of volume, viability and cell count of injectate used to transfer syngeneic tumor into large populations of identical aged syngeneic mice, it is possible to obtain a large (200-500) animal population growing uniform extremity sarcomas. By amputating subgroups of these animals at serial intervals and subsequently assaying pulmonary metastases, it is possible to quantitate the incidence of animals with metastases in the population with the passage of time. By serially assaying splenic lymphocytes from these same animals for DNA synthesis stimulation by phytohemagluttinin (PHA), the time course of nonspecific host immunity can be likewise determined. Similarly, the ability of these animals to reject syngeneic tumor dose challenges and splenic lymphocyte cytotoxicity indices are indicative of antigen-specific immune activity. These have been followed and correlated with the time course of the development of metastases.

Major Findings:

Uniform tumor growth was achieved in these large animal populations through all stages of tumor growth with a standard error of the mean equal to or less than 7%. A characteristic Gompertz function has been found to describe the development of metastases with respect to time with appropriate alpha (log growth) and A (log retarding) factors determinable. This pattern describes four of the murine tumor systems studied. In one however, spontaneous regression of documented pulmonary metastases occurs with the passage of time and the ongoing development of the primary. In all tumor systems, the nonspecific lymphocyte PHA response and the specific ability to reject syngeneic tumor cell challenges correlates with the appearance of metastases. In the tumor system shown to produce spontaneously regressing pulmonary metastases, this immunologic picture is particularly striking.

Significance to Biomedical Research and Program of the Institute

The understanding of the kinetics of the metastatic process is as fundamental to the understanding of metastases as the mathematical approach to tumor growth is to the understanding of the growth of primary tumors. It provides a tool for the quantitation (rather than the heretofore semiquantitation) of the effects of proposed adjuvants. Finally, it provides a quantitative means of comparison with other events such as the development of specific and nonspecific immunity.

Proposed Course:

The studies of the development of the cytotoxic reactivity of splenic lymphocytes of these animals are still underway. This technique can then be applied to the evaluation of anticoagulants and other adjuvants proposed for the prevention of metastatic disease.

Project Description:

Part IV. The Metastatic Potential of Experimental Pulmonary Metastases

Objectives:

To determine the effect of tumor-specific immunity on the propensity for tumor formation by circulating tumor cells shed from metastatic tumors.

Methods Employed:

Two tumor systems were used: An immunogenic MCA-induced tumor and a nonimmunogenic S91 melanoma. C57Bl/6N mice were used with the first tumor and CDF1 mice with the second. The design was identical in both experiments. Mice with previously amputated primary tumor bearing legs and pulmonary metastases were sacrificed and their left heart blood aspirated. The blood was used to inoculate mice pairs intravenously (I.V.). These recipients were designated Group 2 mice. The pairs were divided into two groups, Group 2A and Group 2B. Each of these groups were further divided into two additional groups according to the time of sacrifice after inoculation of blood: Group 2A-1 was sacrificed 24 hours after inoculation, Group 2B-1 was sacrificed one week after inoculation, and Groups 2A-2 and 2B-2 were sacrificed six weeks after inoculation. When Group 2 mice were sacrificed, left heart blood was aspirated and inoculated into normal recipients, designated Group 3. These mice received the same subgroup designations as the Group 2 blood donors. When several mice in Group 3A-2 and 3B-2 died approximately six weeks after inoculation, all Group 3 mice were sacrificed.

Major Findings:

The results were similar with both animal tumor systems studied. In the C57Bl/6N mice inoculated with MCA-induced tumors, no lung tumors were found in Group 2 mice that were sacrificed one day and one week after I.V. inoculation of blood containing circulating tumor cells, nor were any found in Group 3 mice inoculated with blood aspirated from these Group 2 mice. In Group 2, animals sacrificed six weeks after inoculation, a high incidence of lung tumors was produced: 14/18 in Group 2A and 13/16 in Group 2B-2. Also, lung tumors were produced in Group 3 animals inoculated with blood from these mice: 5/10 in Group 3A-2 and 5/11 in Group 3B-2. In the CDF1 mice inoculated with S91 melanoma, Group 2 animals sacrificed on one day and one week after tumor inoculation had no lung tumors, neither did their respective Group 3 blood recipients. On the other hand, a total of 35/42 Group 2 mice had lung tumors when sacrificed six weeks after inoculation, and the blood from these animals produced lung tumors in 15/30 Group 3 recipients. These results showed that blood from mice with pulmonary metastatic tumors readily produced lung tumors when inoculated into normal mice. In Group 2 mice, the possibility existed that the lung tumors produced may have generated from cells shed from the previously amputated primary tumor. However, the subsequent transfer of blood to other mice (Group 3) produced tumors only with blood from Group 2 mice that had large lung tumors, thus demonstrating that their origin is in circulating tumor cells shed from pulmonary tumors, not from the primary tumor. The experiment thus demonstrates that pulmonary metastases may shed cells that survive and produce new tumors, and since the results were the same with an immunogenic tumor as with a non-immunogenic tumor, it demonstrates that cells from metastases of an immunogenic tumor enjoy the same facilitation in their propagation as cells from non-immunogenic tumors.

Significance to Biomedical Research and Program of the Institute:

These observations may have clinical significance to one of the most perplexing problems in clinical oncology - the metastatic potential of tumor metastases. Frequently, in a patient with a controlled primary tumor and a single metastasis deposit, a waiting period is elected to allow for the appearance of additional metastases. This course entails a theoretical danger of allowing the dissemination of cells from the metastases that produce tumors elsewhere. The data give no support to the hypothesis often advanced that immunity may destroy circulating tumor cells. Until data from human experiments show that this phenomenon does not occur in humans, the clinician must consider the experimental evidence which indicates that metastatic deposits shed cells that can colonize elsewhere in the tumor-immunized host.

Proposed Course:

Experiments now in progress are designed to define both the specific and nonspecific serum factors that facilitate the survival and propagation of circulating tumor cells in this experimental model.

Project Description:

Part V. Ultrastructural Features of the Formation of Pulmonary Metastases

Objectives:

The early cellular events in the formation of pulmonary metastases are largely unknown. The mechanisms involved in the entrapment of circulating, blood-borne tumor cells in pulmonary capillaries, the migration of trapped tumor cells from capillaries into lung parenchyma, and the growth of the metastatic nodule itself are all obscure and are objects of much speculation. Reports in the literature are controversial concerning such problems as: whether tumor cell entrapment in pulmonary capillaries is a purely mechanical one or a process related to the adhesive characteristics of the cell membrane; whether fibrin or other plasma proteins play significant roles in tumor cell entrapment; whether only specialized cells in selected areas of lung may give rise to metastases or whether the entire process is completely random; or whether different metastatic tumors behave similarly in the establishment of pulmonary metastatic foci. In order to begin examination of such controversial points, a study was begun of electron microscopic observations of experimental pulmonary metastases in a murine sarcoma system.

Methods Employed:

Dissociated cell suspension of methylcholanthrene-induced fibrosarcoma were prepared by trypsinization. 1.0×10^6 tumor cells were injected intravenously into groups of C57Bl/6 mice, which were sacrificed at sequential intervals after injection. The lungs were fixed and prepared for both conventional light microscopy and for electron microscopy. Gluteraldehyde-fixed, Epon embedded tissue blocks were serially sectioned to locate tumor cells, and those areas were thin-sectioned for examination under the electron microscope.

Major Findings:

Circulating tumor cells were present in pulmonary capillaries within 15 minutes of injection. Such cells often had extensive surface pseudopodia, with which they attached to pulmonary endothelial cell membranes within 90 minutes. Tumor cell attachment was followed by massive platelet aggregation which resulted in the formation of a platelet thrombus which often completely filled the pulmonary capillary and its afferent arteriole. No fibrin was present in the platelet thrombus. The platelet thrombus disintegrated in 18 hours, but tumor cells continued to adhere to endothelial cell membranes by characteristic surface projections. Tumor cells then penetrated the capillary wall through the endothelial junctional complex regions. a process most frequently observed between 24 and 72 hours following tumor cell infusion. Tumor cells, once within the pulmonary parenchyma, underwent division to form cell nests, which developed into macroscopic metastases in all experimental animals within 7 days.

Significance to Biomedical Research and Program of the Institute:

Understanding the sequence of cellular events in the establishment of pulmonary metastases could lead to attempts at the development of methods for interrupting the sequence at a variety of levels. Such methods of blocking steps in the formation of metastases could have therapeutic implications in human tumors.

Proposed Course:

Similar series of observations to be made on different classes of metastatic murine tumors such as melanoma and lymphoma. Experimental observations will be repeated in the presence of experimentally altered blood coagulability and platelet activity in the presence of anticoagulants and platelet inhibitors.

Publications:

Ketcham, A.S., Wexler, H. and Chretien, P.B.: The metastatic potential of experimental pulmonary metastases. J. Surg. Res. 15:45-52, 1973.

Serial No. NCI-3806
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Surgical Physiology and Metabolic Changes Associated with Extensive Cancer Surgery

Previous Serial No.: Same

Principal Investigators: R. M. Beazley, M.D. and R. C. Hoye, M.D.

Other Investigators: G. W. Geelhoed, M.D., S. H. Bennett, M.D., R. K. Aaron, M.D., J. P. Grant, M.D., L. Schour, M.D., G. B. Bulkley, M.D., D. H. Bagley, M.D. G. C. Riggle, B.S., and C. Gorschboth, B.S.

Cooperating Units: Anesthesiology Department, CC, Blood Bank Department, CC, Pharmacy Department, CC, and Biomedical Engineering and Instrumentation Branch, DRS.

Man Years:

Total:	7
Professional:	4-1/2
Others:	2-1/2

Project Description:

Part I: Application of Total Parenteral Nutrition to Postoperative Cancer Patients

Part II: Effects of Autotransfusion on Blood Elements and Pulmonary Function

Part III: Surgical Stress and Coagulation

Part IV: Measurement of Hepatic Blood Flow in Primates

Part V: The Influence of Abdominal Surgery on Gastric Emptying

Project Description:

Part I. Application of Total Parenteral Nutrition to Postoperative Cancer Patients

Objectives:

The importance of adequate nutrition in the promotion of would healing and resistance to infection has long been known. The availability of hypertonic dextrose solutions with protein hydrolysates now provides a technique whereby adequate nutrition can be maintained in patients unable to tolerate an oral diet. A protocol was developed to standardize the application of total parenteral nutrition to postoperative patients and to study the metabolic effects on the stressed patient.

Methods Employed:

A registrar has been established to record insertion of subclavian catheters and complications of a non-infectious and infectious nature. Patients are screened prior to surgery for kidney and liver function and their metabolic status assessed by complete blood chemistries. During therapy patients are followed at weekly intervals with repeat blood studies and 24 hour urine collections to determine a daily metabolic balance.

Major Findings:

No complications from placement of 22 subclavian catheters have occurred in 15 patients. Cultures of catheter tips and blood cultures through the catheter at the time of removal revealed no primary catheter infections. Total parenteral nutrition has been given from 2 to 35 days with an average of 12.9 days. In all patients studied a positive nitrogen balance was obtained with gradual weight gain. No major electrolyte complications occurred as patients were followed closely and corrections made immediately. An inadequate number of patients have been followed to determine if there is a decrease in morbidity and hospital stay with total parenteral nutrition, but to date no increased morbidity has resulted. Three patients have received essential amino acids and hypertonic dextrose solutions for treatment in two of renal failure and in one of hepatic failure. In both cases of renal failure, blood urea nitrogen was found to decrease and stabilize and serum potassium returned to normal. In the patient with hepatic failure, blood ammonia levels decreased rom 2-3 times normal and stabilized slightly above normal.

Significance to Biomedical Research and Program of the Institute:

It is felt when properly monitored that total parenteral nutrition is a safe procedure in postoperative cancer patients and offers an early return to positive nitrogen balance and subsequent benefits of early wound healing and more rapid recovery. The utilization of essential amino acids and hypertonic dextrose solutions provide a useful mode of therapy for the hypercatabolic patient with borderline to failing renal function in the treatment of elevated blood urea nitrogen and potassium. The use of essential amino acids in hepatic failure will need more research before any general conclusions can be drawn.

Proposed Course:

Continuation of clinical studies and development of animal experimental model to further evaluate essential amino acids and liver function.

Project Description:

Part II. Effects of Autotransfusion on Blood Elements and Plumonary Function

Objectives:

1) To determine the pulmonary function of animals subjected to intraoperative autotransfusion of 2-3 times their blood volume, and 2) to examine the chemical and morphological characteristics of blood after it has been passed through an autotransfusion device.

Methods Employed:

The Bentley intraoperative autotransfusion device and broad suction apparatus developed in this laboratory were studied for their effect on postoperative pulmonary function in dogs. These animals were subjected to autotransfusion of 2-3 times their estimated blood volume following which various parameters of ventilation and pulmonary mechanics were studied. These included the compliance to functional residual capacity ratio, arterial venous O_2 difference, O_2 uptake, CO_2 production, alveolar ventilation, dead space, the VD_2 to VT ratio, arterial alveolar gradient, pulmonary shunt, pulmonary arterial pressure, pulmonary capillary wedge pressure and pulmonary vascular resistance. In addition, the cardiac output and stroke volume were measured and expressed in cc's per minute per kilogram and cc's per kilogram respectively.

Blood was collected from the operative wound at the time of surgery in 10 patients undergoing extensive resection of malignancy and compared with 10 units of homograft whole bank blood. Morphological integrity was examined by studying the hemoglobin, hematocrit, red blood count, white blood count, platelets and serum free hemoglobin and screen filtration pressure of these 20 units of blood. In addition, the pH, pO_2, pCO_2, sodium, potassium, chloride, CO_2 and calcium were measured. The enzymes SGOT, SGPT, LDH, AND CPK were studied as were the direct and total bilirubin.

Major Findings:

The pulmonary function in the control group of animals remains stable throughout the operative period. In those animals who subjected to intraoperative allotransfusion of fall in hemoglobin and a rise in screen filtration pressure were observed along with a metabolic acidosis. The arterial venous 0_2 difference was transiently depressed postoperatively, but rapidly returned to within normal limits. Pulmonary dead space in the VD to VT ratio increased significantly in the postoperative period. The arterial alveolar gradiant increased significantly in the postoperative period and persisted through the 48 to 72 hour study. The percent venous admixture likewise increased; however, the most striking abnormalities were a rise in pulmonary arterial pressure and a rise in pulmonary vascular resistance which persisted until 72 hours after autotransfusion.

In comparing autotransfused and human bank blood, several striking abnormalities were noted. Both contain an unacceptably high level of microagregates when passed through standard blood administration filters. Bank blook is not a good source of platelets. Conversely, marked hemolysis occurred in allotransfused blood with a mean plasma-free hemoglobin level in 1,000 milligrams percent. Salvageable red cell mass was quite low. Blood collected by the allotransfusion device was more favorable in the electrolyte composition. Bank blood samples demonstrated somewhat higher levels of sodium and potassium. Bank blood samples were very acidotic with the mean pH of 6.63 and the mean PCO_2 of 158 millimeters of mercury. A hypocarbic alkalosis was observed in allotransfused blood. However, a massive shift in pH was prevented by diminution of the total CO_2 and the pH was much closer by 0.5 pH units to the ideal 7.42.

Significance to Biomedical Research and Program of the Institute:

Perfection of an autotransfusion system will have direct human surgical application. It will enable more extensive surgery to be done without complete dependence upon normal bank blood.

Proposed Course:

Further work needs to be done in perfecting the allotransfusion device, especially with respect to red cell damage. This is essential to provide as safe an alternative as bank blood in the problem of restoring blood volume and red cell mass during surgery.

Project Description:

Part III Surgical Stress and Coagulation

Objectives:

To determine the relationship of surgical stress and coagulation on the development of postoperative venous thrombosis, interaction of the coagulation mechanisms and kinin generation in the production of the postoperative inflammatory response.

Methods Employed:

Patients were studied preoperatively, intraoperatively and postoperatively with respect to prothrombin times, partial thromboplastin time, template bleeding times, Factor 12, Factor 8, and glass and silicone clotting times. In addition, the prekallikrein and kininogen levels of these patients were determined.

Major Findings:

There appears to be a transient depletion of Factors 8 and 12 intraoperatively persisting for the first to second postoperative day with rise toward normal levels and occasionally a rebound in the latter postoperative period. These changes were reflected only partially in the partial thrombinplastin time and not at all in the prothrombin time. Template bleeding times were accelerated in the postoperative period. Depletion of prekallikrein and kininogen was observed intraoperatively and for the early postoperative periods suggesting activation of the kinin precursor system. These findings are consistent with the clinical observation of the appearance of postoperative inflammation and coagulative challenge.

Significance to Biomedical Research and Program of the Institute:

These studies serve to provide laboratory basis clinical understanding of the relationship between surgical stress, blood coagulation and inflammation.

Proposed Course:

These studies should be continued in order to better define the role of the intrinsic coagulation system in surgical stress and to search for an index of impending deep vein thrombosis. Understanding of the postoperative inflammatory response is enhanced by an appreciation of kind and activation during surgery.

Project Description:

Part VI Measurement of Hepatic Blood Flow in Primates

Objectives:

The goal of this project was to devise a surgical model for chronically studying hepatic blood flow in primates. The wash-out of radioactive xenon from the hepatic vascular bed was to be compared with the standard method of BSP excretion for determining total hepatic blood flow. The contribution to total hepatic blood flow by the hepatic artery and protal vein was to be determined. The effect of gastrointestinal hormones on blood flow to the liver was to be studied.

Methods Employed:

Rhesus monkeys and adult baboons were prepared in the following manner: A splenectomy was performed. Small silastic cathethers were inserted into the hepatic artery and portal vein. These were brought through the abdominal wall and occluded with a blunt needle having a rubber diaphragm over the end. This blunt needle was sutured to fascia. Needles were placed through the skin into the blunt needles for injection of xenon or other study materials. The hepatic artery was isolated on the celiac access by ligating the left gastric, the pancreaticoduodenal, the cystic, and the right gastric arteries. An electromagnetic flow meter was placed around the hepatic artery. A pneumatic occluder was also placed downstream to the electromagnetic flow meter. A third silastic catheter was placed in the hepatic vein.

Total hepatic blood flow was determined by BSP excretion during constant infusion. Hepatic blood flow was also determined from the radioactive xenon washout curves over the liver. The hepatic artery and portal vein sites were used for injection of xenon 133 and the flows from each compared to that calculated by BSP excretion. Hepatic artery blood flow was measured by the electromagnetic flow meter. Pitocin and glucagon were injected into the animal at different times and hepatic blood flow as measured by xenon-133 washout and the electromagnetic flowmeter were compared.

Major Findings:

The surgical model for studying hepatic blood flow has been successful in allowing us to study primates multiple times over a 10-14 day period. Total hepatic blood flow as measured by BSP excretion (523±155 ml/min) compares very favorably with hepatic blood flow as measured by xenon-133 washout curves when the xenon was injected via the portal vein (509±158 ml/min). Pitocin had the effect of decreasing total hepatic blood flow and glucagon increasing total hepatic blood flow, both to a significant amount. In both instances, the hepatic artery seemed to recover more quickly from the effect of these two drugs as compared to the portal vein blood flow.

Significance to Biomedical Research and Program of the Institute:

A method for evaluating total hepatic blood flow in patients would be very helpful in evaluating patients with liver disease prior to undergoing surgery. An understanding of the flow patterns within the liver may also be of use in designing chemotherapy for liver metastatic disease.

Proposed Course:

This project will be continued with the hope of developing a non-traumatic method for estimating total hepatic blood flow in humans. Other gastrointestinal hormones or active peptide fractions that have effect upon the gastrointestinal system will also be studied to determine their effects upon total hepatic blood flow.

Project Description:

Part V The Influence of Abdominal Surgery on Gastric Emptying

Objectives:

Postoperative ileus is a common surgical problem. By developing a method to quantitatate gastric emptying as a measure of general gastrointestinal motility a means for exploring the underlying mechanisms and therapeutic approaches would be provided.

Methods Employed:

A computer-based mathematical model of the individual components of the gastric emptying process has been established in animals. This model has also proved to be accurate when applied to humans. Using this model, based on volume computations derived from resultant dilutions of a serially added volume/concentration marker a normal postoperative pattern has been established in patients undergoing non-gastrointestinally massive exploratory laparotomy. This same technique will also be used to evaluate the role of alpha and beta sympathetic blocking agents in the prevention of postoperative gastric atony.

Major Findings:

The validity of the above method and mathematical model has been confirmed in a group of normal volunteers. The method was then applied to operative patients and has established a characteristic and striking pattern of gastric hypomotility that is maximum immediately after surgery, deminishing significantly by the third postoperative day. A sinusoidal pattern representing a delayed negative feed-back system that is seen in the normal patient is almost completely abolished immediatively postoperatively.

Significance to Biomedical Research and Program of the Institute:

To quantitate postoperative ileus is to enable one to eventually evaluate agents for its control. Such control would represent a significant benefit to patients undergoing major cancer surgery.

Proposed Course:

Following final analysis of the control studies, a protocol will be proposed for the investigation of alpha and beta sympathetic blocking agents for the treatment of postoperative ileus.

Publications:

Bennett, S.H., Geelhoed, G.W., Terrill, R.E., and Hoye, R.C.: Pulmonary effects of autotransfused blood retrieved from the pleural cavity in an *in situ* perfusion model. *Amer. J. Surg.*, 125: 696-702, 1973.

Bennett, S.H., Geelhoed, G.W., Gralnick, H.R., and Hoye, R.C.: Effect of autotransfusion on blood elements. *Amer. J. Surg.*, 125: 273-279, 1973.

Joseph, W.L., Rivera, R.A., O'Kieffe, D.A., Geelhoed, G.W., and McCune, W.S.: Management of postoperative alkaline reflux gastritis. *Ann. Surg.*, 177: 655-659, 1973.

Larson, S.M., Millar, R.C., Chalmers, T.C., Gelrud, L.G., Kramer, R.J,, Bailey, J.J., and Johnston, G.S.: Quantitation of hepatic blood flow by Xenon-133 clearance: A Primate Model. *Gstaad Symposium*, 1: 96-106, 1973.

Serial No. NCI-3815
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Transplantation of the Parathyroid Glands

Previous Serial No. : Same

Principal Investigator: S.A. Wells, Jr., M.D.

Other Investigators: C. Christiansen, B.S.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Others:	1/2

Project Description:

Transplantation of the Parathyroid Glands

Objectives:

To determine the environmental factors involved in transplantation of parathyroid glands.

Methods Employed:

Utilizing highly inbred Fischer strain rats, parathyroid glands were divided into three groups. In one group, the parathyroid glands were dried in situ on the muscle bed after being removed for 1, 3, and 6 hours respectively. They were then implanted in the thigh muscle of a hypoparathyroid recipient. In a second group, the parathyroid glands were removed and placed in Waymouth media at room temperature for 3, 6, 9, and 12 hours following which they were implanted in hypoparathyroid recipients. After implantation calciums were monitored. At 14 days the grafts were removed and sent for histology. In a third group of animals the glands were removed and frozen for periods of 3, 6, 9, and 12 months respectively. Glands were then reimplanted and serum calciums were monitored being removed after 14 days for histology. All glands, regardless of the method of treatment, survived and were histologically and physiologically indistinguishable from fresh normal transplanted parathyroid tissue.

Significance to Biomedical Research and Program of the Institute:

During the course of surgery for certain thyroid cancers as well as other primary cancers of the neck, it may be necessary to remove all parathyroid glands with adjacent tumor tissue. Fundamental factors involved in grafting of parathyroid tissue must be completely understood in order to determine the conditions under which reimplantation may be undertaken following either intentionally or inadvertent excision during the course of cancer surgery.

Proposed Course:

Further investigations should be carried out to develop the best techniques for parathyroid transplantation to insure optimum growth of these transplants.

Publications:

S.A. Wells, Jr., Christiansen, C.: The transplanted parathyroid glands: Evaluation of cryopreservation and other environmental factors which affect its function. Surgery 75: 49-55, 1974.

Serial No. NCI-3816
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NCI
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cryobiology

Previous Serial Number: Same

Principal Investigator: R.M. Beazley, M.D.

Other Investigators: D.H. Bagley, M.D., H.B. Neel, III, M.D. and A.S. Ketcham, M.D.

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years:

Total:	1-1/2
Professional:	1/2
Others:	1

Project Description:

Part I. Effect of Cryodestruction on the Primate Facial Nerve

Part II. Effect of Cryodestruction on the Canine Recurrent Laryngeal Nerve

Part III. Experimental Evaluation of In Situ Oncocide for Primary Tumor Therapy: Comparison of Tumor Specific Immunity After Complete Excision, Cryonecrosis and Ligation

Project Description:

Part I. Effect of Cryodestruction on the Primate Facial Nerve

Objectives:

To determine the effect on structure and function of primate facial nerve which has been subjected to cryodestruction.

Methods Employed:

The Linde CE2, PR-5 probe was modified so that it could be mounted and held in an operating microscope stand. Utilizing microminiature thermocouples and a high speed 6-channel recorder, cryolesions were made on surgically exposed primate facial nerve.

Major Findings:

Complete ipsilateral facial palsy has been produced in all animals. Gradual return of function was monitored by serial motion picture recordings for the next six to eight weeks. Complete and normal nerve function resumed in all animals within eight weeks.

Significance to Biomedical Research and Program of the Institute:

The ability of the facial nerve to regain function after a segment of the nerve has been destroyed by cryodestruction has direct application in human tumor surgery. This may enable the surgeon to ablate inoperable tumors with cryosurgery while saving structure and function of important nerves in the immediate tumor area.

Proposed Course:

Future studies will be expanded to standardize the degree and extent of injury to determine maximally tolerable temperature depression of nerves that is compatible with functional recovery.

Project Description:

Part II. Effect of Cryodestruction of the Canine Recurrent Laryngeal Nerve

Objectives:

To determine the effect on structure and function of the canine recurrent laryngeal nerve which has been subjected to cryodestruction.

Methods Employed:

The Linde CE2, PR-5 probe was modified so that it could be mounted and held in an operating microscope stand. Utilizing microminiature thermocouples and a high speed 6-channel recorder, cryolesions were made on the surgically exposed canine recurrent laryngeal nerve.

Major Findings:

Complete ipsilateral recurrent nerve palsy, with resultant vocal cord paralysis was noted in all animals thus treated. The return of function was monitored by repeated direct laryngoscopies over the following six to eight weeks. 90% of animals thus treated regained normal cord function within eight weeks.

Significance to Biomedical Research and Program of the Institute:

The ability of recurrent laryngeal nerve to return to function after undergoing cryodestruction may have widespread application in oncological surgery.

This will enable the surgeon to ablate tumor tissue which may encroach on the nerve, and at the same time, save structure and function of the nerve and its innervated tissue.

Proposed Course:

Studies in progress will define tolerance of recurrent nerve to temperature depression and repetitive freezing.

Project Description:

Part III. Experimental Evaluation of In Situ Oncocide for Primary Tumor Therapy: Comparison of Tumor Specific Immunity After Complete Excision, Cryonecrosis and Ligation

Objectives:

Test the hypothesis that in situ tumor cell necrosis, in addition to being a feasible means of curing primary tumors, increases host response against the tumor immunological mechanisms. A typical mammary adenocarcinoma and a methylcholanthrene induced sarcoma were utilized in mice. After the tumors had reached approximately 1 cm in diameter, they were treated in the following ways: One group was completely surgically excised, one group was frozen using standardized cryosurgical technique, and the third group was treated by infarction of the tumor. Those animals which subsequently demonstrated complete cure were challenged five weeks later with varying dilutions of the same tumor cell suspension.

Major Findings:

In both the sarcoma group and in the breast carcinoma group, higher tumor challenge doses were needed to induce recurrent growth in those animals frozen as compared to those animals which underwent surgical excision. The immunological effect of freezing was greater than noted following ligation.

Significance to Biomedical Research and Program of the Institute:

The fact that in situ tumor necrosis produces greater immunity than surgical excision provides an experimental basis in immunology for the clinical results reported after cryosurgical destruciton of various neoplasms. It also suggests that further investigations of in situ oncocide for treatment of surgically unresectable primary and metastatic tumors warranted for potential immunological effects.

Proposed Course:

Expanded investigations of the effects of freezing on host reactions to tumors in experimental animals are in progress. These will be oriented toward possible clinical applications in patients with inoperable tumors.

Publications:

Riggle, G.C., Bagley, D.H., Jr., and Beazley, R.M.: Microthermocouple probe for gradient temperature measurements. Cryobiology, 10: 345-346, 1973.

Bagley, D.H., Beazley, R.M. and Ketcham, A.S.: Effects of cryosurgery on the facial nerve in monkeys. Surg. Forum, Vol. XXIV, 514-516, 1973.

In Press:

Beazley, R.M., Bagley, D.H. and Ketcham, A.S.: The effect of cryosurgery on peripheral nerves. J. Surg. Res.

Serial No. NCI-3819
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of Cancer Immunology and Immunotherapy in Laboratory Animals

Previous Serial Number: Same

Principal Investigators: L. Schour, M.D. and N. Javadpour, M.D.

Other Investigators: M.S. Soloway, M.D., J.W. Futrell, M.D. and R.P. Faraci, M.D.

Cooperating Units: Laboratory of Pathology, National Cancer Institute

Man Years:

Total:	4-1/2
Professional:	2
Others:	2-1/2

Project Description:

Part I. The Influence of Regional Lymphatics on Tumor Immunity

Part II. Regional Lymphadenectomy and the Role of Regional Lymphatics in Cancer Immunity

Part III. Preferential Splenic Metastases in Parabiotic Animals

Part IV. Study of Cancer Immunology and Immunotherapy in Laboratory Animals

Part V. Transformation of Hamster Urothelium by SV40 and Its Possible Prevention and Treatment by Fetal Antigens

Project Description:

Part I. The Influence of Regional Lymphatics on Tumor Immunity

Objectives:

The regional lymphatics are known to play an important role in rejection of skin allografts as demonstrated by experimental work using alymphatic skin pedicles in guinea pigs. Skin allografts on such pedicles will survive indefinitely. Animals with a pedicle graft in place will reject another graft from the same donor in a first-set fashion. Not only is the second graft rejected, but the first graft on the pedicle is also rejected, thereby establishing the presence of intact efferent arc of the immune system. An alymphatic pedicle will be used to study the effect of regional lymphatics on tumor allografts and tumor isografts.

Methods Employed:

Two methylcholanthrene tumors induced in strain 2 guinea pigs will be used as allografts and isografts. These will be injected into strain 2 and strain 13 inbred animals. Alymphatic skin pedicles in each animal will be challenged intradermally with various tumor cell doses. The same model will be used to study immunotherapy and adoptive transfer of tumor immunity in guinea pigs.

Major Findings:

Tumor allografts injected into alymphatic skin pedicles grew to 3 cm., but all eventually regressed. Tumors grew considerably larger in the pedicles than did tumors resulting from allogeneic tumor cell injection into intact skin. Animals previously bearing a tumor allograft demonstrated immunity upon rechallenge. This immunity could be transferred to other animals by the use of spleen cells. In contrast, tumor isografts in alymphatic pedicles grew to 6 cm in size resulting in death of the animal. This occurred with a cell dose which never produced progressively growing tumor in the intact skin. When a pedicle bearing a tumor isograft was excised early in the course of its growth, subsequent rechallenge of the animal with syngeneic tumor demonstrated immunity.

Significance to Biomedical Research and Program of the Institute:

Some investigators have advocated leaving local lymph nodes in place during the course of cancer surgery in order to preserve the patient's immune defense. Findings in this study suggest that regional lymphatics are important for the early initiation of tumor growth, but that once this tumor obtains a certain size, it is no longer under the influence of the local immune system. Once a tumor is large enough to be clinically detected, our findings suggest that the patient has already been sensitized systemically and would not benefit by leaving the local lymph nodes intact, especially if these may contain metastatic tumor.

Proposed Course:

Further investigations will be carried out using the alymphatic pedicle in guinea pigs to better understand the role of regional lymphatics in tumor growth as well as the effect of surgical manipulation of regional lymphatics in tumor-bearing animals.

Project Description:

Part II. Regional Lymphadenectomy and the Role of Regional Lymphatics in Cancer Immunity

Objectives:

Increasing importance has been attached to the role of the regional lymph nodes in the expression of a host's ability to resist tumor growth. Some have suggested that regional lymphadenectomies significantly alter the host's ability to mount an immune response to a tumor challenge. Studies with certain murine tumor systems have suggested that perhaps patients undergoing surgery for cancer should have their local lymph nodes left intact so that their immune response to tumor is not significantly altered. The purpose of this study was to determine what affect regional lymphadenectomy would have upon the host's ability to develop concomitant immunity to tumor-specific transplantation antigens.

Methods Employed:

Two hundred and twenty inbred Balb/c mice were randomly inoculated in the right hind limb with $1x10^5$ viable isologous fibrosarcoma cells from a methylcholanthrene-induced tumor. Twenty mice served as unoperated controls. The remaining 200 mice were divided into four groups of 50 mice each and challenged with $1x10^4$ tumor cells in the left hind limb on days 3, 7, 14 and 21 after initial right limb challenge. Prior to the second tumor challenge, 20 mice underwent right popliteal and inguinal lymphadenectomy and 10 mice underwent sham operation. The remaining 20 mice served as controls with 10 additional mice serving as controls for the tumor cell dose used for each challenge.

Major Findings:

Significant concomitant tumor immunity was demonstrated by failure of the second tumor challenge to grow (0-10%) in those mice whose lymphadenectomy was performed on days 7, 14 and 21 as well as in the sham operated and non-lymphadenectomized controls. In contrast, a significant lack of concomitant immunity occurred in those mice whose lymphadenectomy was done on day 3. Forty-two percent of this group grew left and right limb tumors.

Significance to Biomedical Research and Program of the Institute:

This study, along with other studies in guinea pig alymphatic skin pedicles, suggests that although regional lymph nodes are important for early host recognition of tumor antigens, their later removal does not adversely alter the host's ability to mount a tumor-specific immune response.

Proposed Course:

Studies will be continued to further clarify the role which the regional lymph nodes play in the host's response to this tumor.

Project Description:

Part III. Preferential Splenic Metastases in Parabiotic Animals

Objectives:

Experiments have been carried out with a spontaneously arising reticulum cell sarcoma which spreads preferentially to spleen. Studies were carried out to see if this tumor would metastasize across a parabiotic union and to determine if the splenotrophism of this tumor would be manifest in parabiotic animals.

Methods Employed:

Normal C3H female mice or splenectomized mice were placed in parabiosis with normal C3H mice. Fourteen days later, the splenectomized animal or a normal animal of a normal-parabiotic pair received 10^6 reticulum cell sarcoma cells intramuscularly. Twenty-one days later, animals were sacrificed and examined for presence of gross metastatic tumor. In a second experiment, the single animals (control or splenectomized) received 10^6 cells intramuscularly. Other animals received 10^6 cells intramuscularly and were then placed in parabiosis either with normal animals or with splenectomized animals. Twenty-three days later, animals in all groups were sacrificed and examined for the presence of metastatic tumor.

Major Findings:

Injection of tumor cells into animals in parabiosis for 14 days was followed by splenic metastases in 100% of the animals. There were no extra splenic metastases. Injection of tumor cells into the splenectomized member of a pair of animals placed in parabiosis 14 days earlier, produced splenic metastases in 100% of animals with no extra splenic metastases. Normal single animals receiving tumor cells showed splenic metastases in 100% with metastases to the liver in 36% and to the kidney in 9%. The incidence of metastases in splenectomized animals was 93% to liver and 61% to kidney. When normal animals were injected with tumor cells and then placed in parabiosis with another normal animal, splenic metastases were found in all animals with metastasis to the liver in 2 of 12 injected animals and metastasis to the kidney in 1 of 12 injected animals. When a single animal was injected with tumor cells and then placed in parabiosis with a splenectomized animal, tumor was found in the spleens of 100% of the injected animals and 100% of the uninjected member of the pairs. No extra splenic metastases were seen in the injected member of this pair, but liver metastases occurred in 22% of the uninjected animals and 11% kidney metastases were seen in this group.

Significance to Biomedical Research and Program of the Institute:

These studies demonstrate that metastases of a reticulum cell sarcoma induced by injection of tumor cells intramuscularly in one animal, can spread across a parabiotic union to involve a second animal. The marked splenotrophism of this tumor results in selective metastasis to the spleen of a normal animal placed in parabiosis with a tumor-bearing animal. The splenotrophism of this tumor, as well as its ability to be transmitted across a parabiotic union, should provide an excellent model for the study of immunologic factors influencing preferential metastases.

Proposed Course:

Various methods of altering the immunoresponsiveness of normal animals placed in parabiosis with animals bearing this splenotrophic tumor will be studied. Immunosuppression by x-irradiation and cortisone administration will be carried out in one or both members of the parabiotic pairs.

Project Description:

Part IV Study of Cancer Immunology and Immunotherapy in Laboratory Animals

Objectives:

The specific inhibition of murine melanoma growth in Balb/C Mice pretreated with BCG injections was subjected to in vitro study. Purpose of this study was to determine effects of prior immunization with BCG on lymphocyte mediated cytotoxicity.

Methods Employed:

Balb/C mice underwent intramuscular and intraperitoneal injections of BCG. Control and BCG treated mice were then divided into three groups. One group was challenged with S-91 melanoma cell line, the second group received challenge with a murine sarcoma cell line and the third group was challenged with a spontaneously arising mammary carcinoma line. Tumor incidence was observed in all animals at weekly intervals following inoculation. Lymphocytes were harvested from animals challenged with all three tumors and used in cytotoxicity tests in vitro. Target cells were the original tumor target cells. Lymphocytes harvested from a group of BCG pretreated animals were also tested in vitro against S-91 target cells as well as normal fibroblasts from Balb/C animals.

Major Findings:

Pretreatment with as low as two doses of BCG produced a 70% reduction in melanoma incidence compared with the incidence in controlled animals (P is $< .001$). The same BCG pretreatment did not protect against challenge with 1×10^5 mammary carcinoma cells or 1×10^4 MCA fibrosarcoma cells in the same strain of mice. Lack of immunogenicity was not a factor in the inability of the carcinoma and sarcoma to be inhibited by BCG since the tumors were proven to be highly immunogenic.

Lymphocyte mediated cytotoxicity studies paralleled the results of the in vivo experiment. Lymphocytes immune to each of the three tumors showed significant cytotoxicity against their respective tumor target cells ($P < .001$). Lymphocytes from BCG pretreated animals showed significant toxicity only against the S-91 target cells ($P < .01$). Nonspecific cytotoxicity was ruled out since BCG immune lymphocytes were not cytotoxic to Balb/C fibroblasts.

Significance to Biomedical Research and Program of the Institute:

The decreased tumor incidence in the BCG immune animals when challenged against melanoma as compared to no effect following sarcoma and carcinoma challenge, suggests that there may be specific immunologic factors providing protection by BCG injection against melanoma. These findings are at variance with the commonly accepted idea that BCG immunization acts only through general nonspecific stimulation of the immune system to produce inhibition of tumor growth in animals and humans. There may be specific rejection of melanoma cells due to sharing of common antigens by tubercle bacilli and melanoma.

Proposed Course:

In vivo and *in vitro* studies are underway to investigate the behavior of other tumors in animals pretreated with BCG to attempt to determine further if there is indeed specific BCG protection against melanoma.

Project Description:

Part V. Transformation of Hamster Urothelium by SV40 and its Possible Prevention and Treatment by Fetal Antigens

Objectives:

Since the urothelial cancers are usually multicentric and recurrent, a possible viral etiology has been suggested. Due to the lack of technique to isolate the urothelium from the underlying lamina propria, it has not been possible to culture and transform the urothelium.

Methods Employed:

Inbred hamsters have been used for cystectomies. A new simple technique has been devised to separate the mucosa (urothelium) of the bladder by injection of 1-2 cc of normal saline in the lamina propria and subsequently dissect the urothelium for tissue culture. After the cell lines have been established, the urothelium have been transformed *in vitro* by addition of SV40. It has been planned to transplant the transformed urothelial tissue in syngeneic hamsters to establish the tumor. The fetal antigen prepared from the 10-12 day old embryo has been used to immunize a group of syngeneic hamsters. Attempts will be made to transplant the already established tumor into the "immunized hamsters." A group of hamsters with injection of the plain tissue as well as other groups of hamsters will serve as appropriate controls.

Major Findings:

Thus far, 50% of the urothelium has been transformed by SV40. T-antigen has been present in transformed cells. Preliminary data indicates the presence of cross-reactivity between the antigen of transformed tissue and fetal antigen.

Significance to Biomedical Research and Program of the Institute:

The lack of technique to isolate the urothelium has been a major problem in culturing and transforming the urothelium by oncogenic viruses. As a result, the cross-reactivity and antigenicity of these cancers have not been studied.

Proposed Course:

We will continue to establish the urothelial tissue of the urinary tract in experimental animals; also, the study of cross reactivity between T-antigens of the oncogenic viruses and fetal antigens in order to detect the antigenicity of the tumor and feasibility of immunizing the laboratory animals against these types of cancer.

Serial No. NCI-3820
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Non-Specific and Tumor-Specific Reactivity in Tumor-Bearing Hosts

Previous Serial Number: Same

Principal Investigators: M.H. Cohen, M.D., Ph.D., P.B. Chretien, M.D., E.L. Felix, M.D. and A.M. Cohen, M.D.

Other Investigators: R.B. Herberman, M.D., D. Char, M.D., R. Oldham, M.D., and A.S. Ketcham, M.D.

Cooperating Units: Cellular and Tumor Immunology Section, Laboratory of Cell Biology, NCI

Man Years:

Total:	3-1/2
Professional:	1-1/2
Other:	2

Project Description:

Part I.	Effect of Delayed Hypersensitivity Reactions on Lymphocyte Reactivity
Part II.	Cellular Immunity and Blocking Factors in Tumor-Bearing Hosts
Part III.	Cell and Serum Mediated Cytolysis In Vitro and Its Correlation with Immunity

Project Description:

Part I. Effect of Delayed Hypersensitivity Reactions on Lymphocyte Reactivity

Objectives:

To study the effect of an ongoing delayed hypersensitivity reaction on lymphocyte responsiveness to a "non-specific" stimulus. To develop methods for augmentation of immunotherapeutic potency.

Methods Employed:

Mice, guinea pigs, monkeys and humans were treated with BCG, DNCB or nitrogen mustard, alone or in combinations. In the animal systems, the agents were injected intradermally. The DNCB was applied topically in patients. At frequent intervals thereafter, lymphocytes were tested for reactivity against phytohemagglutinin (PHA), 200,000 lymphocytes per well were incubated for 48 hours with 10 micrograms of PHA or PPD, followed by a 24-hour incubation with 2 microcuries of tritiated thymidine. Then liquid scintillation counting was performed to determine the number of disintegrations per minute of tritiated thymidine taken up by cellular DNA. This is an index of cell division in response to PHA or PPD stimulation.

Major Findings:

Guinea pigs treated with BCG had a higher responsiveness to phytohemagglutinin than did guinea pigs not treated with BCG. The same was true for reactivity against PPD. In addition, guinea pigs treated with BCG, DNCB and nitrogen mustard had the highest reactivities to PHA and PPD. Similarly, increased PHA reactivity of lymphocytes was observed in mice inoculated subcutaneously 10 days previously with 1 to 2,000 micrograms of DNCB. In monkeys who did not receive BCG, the PHA reactivity ranged from 6.0 to 8.2 (stimulation index). In contrast, the PHA reactivity ranged from 8.5 to 33.0 in monkeys previously treated with BCG. Monkeys who also received DNCB and nitrogen mustard intradermally were found to have an average increase of 106% in the PHA stimulation index and 221% in the PPD stimulation index. In animals not treated with DNCB and nitrogen mustard, the stimulation indices fell an average of 15%. Additional treatment with DNCB, BCG and nitrogen mustard gave an average increase of 133% over the immediately previous values and 190% over the initial values for PHA stimulation. In patients with or without previous BCG treatment, the topical application of 2,000 micrograms of DNCB substantially increased the PHA reactivity of circulating lymphocytes.

Significance to Biomedical Research and Program of the Institute:

These studies support the conclusion that agents which produce a delayed hypersensitivity reaction increase PHA responsiveness of the host circulating lymphocytes. This enhancement of peripheral lymphocyte reactivity may be a primary pathway by which BCG is effective in tumor immunotherapy. As in chemotherapy, the combination of agents seems more effective than either agent alone.

Proposed Course:

These studies raise the implication that "combination therapy" may be more effective than "single-agent immunotherapy". Also, the fact that chemical agents are able to produce the same *in vitro* changes as BCG, raises the possibility that these agents may be used in lieu of BCG in selected circumstances. This would avoid the possibility of disseminated infection in BCG-treated patients. The effectiveness of the chemical agents in preliminary experiments in mice, guinea pigs and humans, do not indicate a superiority of BCG over the chemical agents mentioned above. Immunotherapy experiments are planned to further explore the mechanism of anti-tumor effect seen with these agents.

Project Description:

Part II. Cellular Immunity and Blocking Factors in Patients with Skeletal and Soft Tissue Sarcomas, etc.

Objectives:

The main purpose of these studies was to evaluate the presence and degree of cell mediated immunity to human sarcomas and to measure the levels of blocking factors in the serum of patients in various stages of disease.

Methods Employed:

Patients were investigated who were undergoing surgery or had previously undergone surgery at the Surgery Branch. Lymphocytes were tested against a human osteogenic sarcoma cell line and against a fibroblast line control. A human lung epidermoid carcinoma line was also used as a control. In addition, sera from patients in various stages of disease were tested for ability to inhibit cell-mediated cytotoxicity.

Major Findings:

Sarcoma-specific cellular cytotoxicity was observed in patients with skeletal and soft tissue sarcomas. Lymphocytes were cytotoxic to the osteosarcoma cell line but not to the fibroblast lines. This may be due in part to the fact that the fibroblast lines are more resistant to cytotoxic effector cells and serums. Lymphocytes from melanoma patients did not react as well, however. The serum from sarcoma-bearing patients was found to inhibit the sarcoma-specific cellular cytotoxicity. The presence of inhibitory serum in a sarcoma patient is usually associated with recurrent or metastatic disease.

Significance to Biomedical Research and Program of the Institute:

Skeletal and soft tissue sarcomas carry tumor cell surface antigens which may be shared between different patients. The main difference between sarcoma-free and sarcoma-bearing patients seems to be the inhibitory effect of serum from the tumor-bearing patients. An understanding of these factors may help to provide effective therapy to these patients.

Proposed Course:

Attempts should be made to correlate the presence of cell-mediated immunity and "blocking factors" with the patient's clinical course. Attempts to enhance the reactivity of these lymphocytes and to diminish the activity of "blocking factors" should have a beneficial and therapeutic effect. In addition, it should be possible to monitor changes in these modalities with immunotherapy, surgery or other therapy.

Project Description:

Part III. Cell and Serum Mediated Cytolysis In Vitro and Its Correlation with Immunity

Objectives:

To develop a quantitative assay for the measurement of cell-mediated immunity using I-125 IUDR to label target-cell DNA. By utilizing different techniques for assay of cell-mediated immunity, it is anticipated that conclusions may be drawn regarding similarities or differences between the various techniques measuring *in vitro* immunity.

Methods Employed:

Target cells were plated out into a microtest plate. Several days were allowed to elapse after which the I-125 IUDR was added to each well individually. The following day, the label was removed and immune lymphocytes or immune serum was added. Twenty-four to 48 hours later, the reactions were terminated and by gamma counting of the amount of label in each individual well, the number of cells left in the well was estimated. The greater the cytotoxicity, the fewer the counts remaining in the target wells.

Major Findings:

Reactivity of patients with melanoma and breast cancer were evaluated by measuring cytotoxic index against melanoma and breast cell lines. There was considerable cross reaction in the sense that melanoma patients' serum and lymphocytes reacted with breast cancer target cells and the converse was also true. The cytotoxicity demonstrated by the lymphocytes seemed to be higher than that demonstrated by the serum. An analysis and the correlation with "non-specific" reactivity against phytohemagglutinin is currently being evaluated.

Significance to Biomedical Research and Program of the Institute:

The mechanism of cytolysis which has been previously evaluated in the Surgery Branch appears to be that target cells are destroyed by direct interaction with immune lymphocytes or immune serum. The degree of *in vitro* immunization of target cells is yet to be determined. This will have to be assessed to help determine the ultimate interpretation of this assay. Also, the relationship of non-specific and tumor-specific assays will be of interest.

Publications:

LeMevel, B.P. and Wells, S.A., Jr.: Fetal antigens cross-reactive with tumor-specific transplantation antigens. Nature (New Biology), 244: 183-184, 1973.

Serial No. NCI-3808
1. Surgery Branch
2. Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of Host Factors Associated with Implantation and Growth of Cancer

Previous Serial Number: Same

Principal Investigator: S.A. Wells, Jr., M.D.

Other Investigators: J.F. Burdick, M.D., A.M. Cohen, M.D., J.P. Grant and B.P. LeMevel, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total:	3
Professional:	2
Other:	1

Project Description:

Part I. Development of In Vitro Assays of Humoral Immunity

Part II. In Vivo Delayed Hypersensitivity Tumor Specific Reactions in Animals and Patients

Part III. Immunotherapy of Solid Tumors

Project Description:

Part I. Development of In Vitro Assays of Humoral Immunity

Objectives:

Tumor specific antigens have been demonstrated in several viral and carcinogen-induced animal neoplasms. The relative contribution of cell mediated and humoral immunity against these tumors has not been elucidated. In order to facilitate studies in this field, it will be necessary to develop multiple sensitive assays of immunity against transformed cells.

Methods Employed:

Three different techniques were used to assay humoral immunity against tumor cells. The isotopic antiglobulin test has been used in the past for characterizing cell surface antigens of murine SV-40 and polyoma tumors. This test has previously been performed with cells in suspension, but the multiple centrifugations and resuspensions which this technique requires have tended to limit the usefulness of this test. An isotopic antiglobulin microassay was developed using tissue culture monolayers with a simplified washing procedure. The test is analogous to the indirect immunofluorescence test in which antibody is reacted with cells and subsequent antiglobulin is added which attaches specifically to the added antibody. In the indirect immunofluorescence test, this last antiglobulin is labelled with fluorocene, but in the isotopic antiglobulin assay, the antiglobulin is labelled with I^{125} and the enumeration procedures are thereby simplified by using this gamma-emitter.

A second assay which was modified for studies in this area was the I^{125} labelled iodoeoxyuridine (IDU) test. This reagent is incorporated into the DNA of target cells and is a useful tag for these cells since the nonspecific release of the label is very low and since the reutilization after cell death is also very low.

The third assay which was modified for use in assaying humoral immunity was the complement fixation assay which depends in this case on the interaction between antibody and tumor specific antigen followed by the binding of complement to the antigen-antibody complex.

Major Findings:

Using the isotopic antiglobulin assay, it was found that antibody against the tumor-specific surface antigens of carcinogen-induced murine sarcomas were detected with high sensitivity. Antibody against the tumor was detected in the serum of animals which have actively growing tumors. Half of the methylcholanthrene-induced tumors that were studied had a common cell surface antigen as detected by this assay.

Using the I^{125} IDU tumor cell label, the microassay for the detection of cytotoxic tumor-specific antibody revealed that small amounts of serum and cells could be utilized to demonstrate serum mediated immunity against methylcholanthrene-induced sarcomas of mice. The assay was simple, sensitive, and apparently objective. The immune serums were highly cytotoxic at high dilutions against the immunizing tumor cells. At low dilutions, the immune serum was only minimally cytotoxic against unrelated methylcholanthrene-induced tumors of mice. This reactivity could represent cross reaction. However, the cytotoxicity could be absorbed from the immune serum only by the tumor cells used for immunization and not by unrelated tumor cells or by normal cells.

Using the micro-complement fixation technique, tumor antigens could be detected against a methylcholanthrene-induced sarcoma of guinea pigs. In these animals, the immunizing cells had been given intradermally. The sera contained a complement fixing antibody that reacted specifically with antigens prepared from the immunizing tumor, but which did not react with antigens from an unrelated methylcholanthrene-induced sarcoma. Similarly, there was no reaction with antigens prepared from normal tissues. A significant complement fixation titer could be detected with antigens prepared from either freshly excised tumor or with antigens prepared from a tissue culture line of the immunizing tumor.

Significance to Biomedical Research and Program of the Institute:

These studies demonstrate a tumor specific antigen in a variety of methylcholanthrene-induced tumors of mice and guinea pigs. Immune serum from these animals is capable of interacting with these tumor cells using this variety of techniques. Cross reaction seems to be more apparent between methylcholanthrene-induced sarcomas of mice than between the methylcholanthrene-induced sarcomas in guinea pigs. These studies emphasize the importance of serum mediated immunity in these systems and emphasize the necessity for monitoring the humoral aspect of immunity in immunotherapy protocols in animals and in humans.

Proposed Course:

These assays will have to be applied to human tumor systems as well as to more animal tumor systems in order to assess the generality of the principles observed. They will constitute useful monitors in immunotherapy protocols. It will be of interest to correlate the clinical course with the changes in the *in vitro* parameters.

Project Description:

Part II. In Vivo Delayed Hypersensitivity Tumor Specific Reactions in Animals and Patients

Objectives:

The delayed hypersensitivity reaction as a measure of cell mediated immunity allows *in vivo* assessment of tumor immunity in both animals and in man. One method of demonstrating tumor immunity *in vivo* has been the elicitation of delayed cutaneous hypersensitivity reactions to intradermal injection of tumor cell extracts. The purpose of these studies was to utilize this technique to assess the nature of immunity in guinea pigs against methylcholanthrene-induced sarcomas, to determine the amount of fetal antigen cross reactivity with this sytem, and to assess in humans the reactivity of patients with carcinoma of the lung and carcinoma of the uterine cervix against appropriate tumor cell extracts.

Methods Employed:

Inbred strain 2 guinea pigs were immunized with irradiated cells of two different methylcholanthrene-induced syngeneic guinea pig sarcomas. They were also immunized against second trimester fetal tissue and then skin tested with tumor membrane extracts and extracts of fetal tissue. These results were correlated with experiments in which the guinea pigs were also challenged with viable tumor cells.

In the human studies, 75 patients with lung cancer and 14 patients with carcinoma of the uterine cervix were tested against standard skin test antigens, as well as DNCB and tumor extracts. As controls, patients with benign lung disease were evaluated. Patients with unrelated tumors were also tested.

Major Findings:

In the guinea pig studies, a cross reaction was observed between fetal antigens in the second trimester fetuses and transplantation antigens of the methylcholanthrene-induced guinea pig sarcomas. Animals immunized against fetal tissue had an increased resistance to tumor cell challenge. The delayed cutaneous hypersensitivity reaction was positive in almost all animals who were immunized with either tumor or fetal tissue and then skin tested against that same antigen. Animals immunized with fetal tissue also had positive skin tests against tumor antigens.

Among patients with lung cancer or cancer of the uterine cervix, about half reacted against either autologous tumor antigen or allogeneic antigen prepared from another tumor of the same histological type. There was a significant incidence of cross reactivity between patients who had cervical cancer, for example, who were being tested with antigen prepared from cervical carcinoma of another patient. The high incidence of cross reactivity suggests the presence of a common antigen in the tumor cells of patients with lung cancer or cervical carcinoma.

Significance to Biomedical Research and Program of the Institute:

The finding that there is a significant incidence of cross reactivity between fetal antigens and tumor antigens in the guinea pig system provides further basis for the use of fetal antigens in immuno-diagnosis and possibly immunotherapy. "Carcinoembryonic antigens" in patients with cancer of the GI tract, cancer of the liver and elsewhere, are now specific enough to warrant further investigation when they are found in patients who may have an occult carcinoma, or possibly a recurrence of carcinoma after a resection. The possibility also exists that by heightening the immune response against antigens of this type, that prophylaxis against tumor growth may be achieved.

The finding in human patients of a high incidence of cross reactivity between patients with a histologically similar cancer (in this case, cancer of the lung and cancer of the cervix), suggests the presence of a common antigen in these cases. This lends further support to the hypothesis that certain human cancers may be produced by a virus which may leave antigens in different cases. The prospects are also raised for using allogeneic tissue for immuno-diagnosis or immunotherapy.

Proposed Course:

In ongoing studies in the Surgery Branch, the assessment of cutaneous hypersensitivity reactions against tumor cell antigens constitutes a valuable technique in patients who can be skin tested against their own tumor on multiple occasions as they are being immunized against that tumor in a concurrent immunotherapy program. The findings reported above will have to be applied in a wider group of animal and human tumor systems to further assess their general applicability.

Project Description:

Part III. Immunotherapy of Solid Tumors

Objective:

To boost the immunological defenses of a patient against his own cancer and thereby enable him to destroy minimum residual disease.

Methods Employed:

Approximately 40 patients with melanoma or sarcoma were treated in the Surgery Branch, National Cancer Institute, by Dr. Donald L. Morton four years ago in a program in which irradiated autochthonous or allogeneic tumor tissue was injected with BCG, and immune and clinical response measured. About two-thirds of these patients were melanoma patients and one-third were sarcoma patients. Among the melanoma patients, some patients who had only cutaneous disease received only intralesional BCG. Two patients with no residual cancer after regional node dissection received only intradermal BCG.

Major Findings:

Of 40 patients with melanoma and sarcoma treated with immunotherapy, six patients with melanoma are alive four years later and one patient with sarcoma is alive. Most of the six patients with melanoma had only cutaneous disease, although the response to intralesional injection of BCG in patients with satellitosis was quite dramatic in two patients. In one case, about 400 nodules were present as in transit metastases, and in another case, about 20 nodules were present. In both cases after inoculation of most but not all lesions, the satellitosis has disappeared and the patients remain free of active disease at the present time. In the other four surviving melanoma patients, the relative contribution of surgery and immunotherapy to their current survival is not determinable. The one patient with sarcoma who was treated with immunotherapy now has metastatic disease. There was an increase in antibody titer against melanoma and against sarcoma in patients with immunotherapy and autologous tumor cells.

Significance to Biomedical Research and Program of the Institute:

These studies have indicated that immunotherapy of melanoma and sarcoma with BCG is a feasible procedure. The toxicity of the BCG was mostly local ulceration and draining in the sites of BCG inoculation. No untoward effects were noted as a result of injection of irradiated tumor cells. In several patients, spiking fevers necessitated the discontinuance of BCG therapy, although it is possible that these were the patients getting the maximum immune response to the agent. The impact on survival in these patients has yet to be assessed. A control study will be necessary to measure this.

Proposed Course:

Protocols have been developed to assess the usefulness of immunotherapy in patients who have a bulky tumor that can be resected and used as a source for autochthonous cells. These cells will then be administered back to the patient with BCG. The cells will be treated with neuraminidase in order to uncover antigenic sites on the cell surfaces. It is hoped that by utilizaing patients with minimal disease that the immunotherapeutic effects can be maximized. Additional joint studies are in progress with the Immunology Branch and the Chemotherapy Group to study melanoma patients who have a minimal amount of residual disease, but who stand a very high likelihood of succumbing from their disease because of the finding of positive regional lymph nodes. It is in patients with minimum amounts of disease that the maximum effects of immunotherapy will probably be seen, and it is to this group of patients that the main thrust of the program will be directed after toxicity studies have been fully evaluated.

Publications:

Burdick, J.F., Wells, S.A., Cohen, A.M. and Ketcham, A.S.: Development of a simplified isotopic antiglobulin assay for study of tumor-specific antibody. J. Surg. Res. 14:200-206, 1973.

Wells, S.A., Jr., Melewicz, F.C., Christiansen, C. and Ketcham, A.S.: Delayed cutaneous hypersensitivity reactions to membrane extracts of carcinomatous cells of the cervix uteri. Surg. Gynec. Obstet. 136:717-720, 1973.

Wells, S.A., Burdick, J.F., Christiansen, C., Ketcham, A.S. and Adkins, M.D.: Demonstration of tumor associated delayed cutaneous hypersensitivity reactions in lung cancer patients and in patients with carcinoma of cervix. Natl. Cancer Inst. Monograph 37:197-203, 1973.

Morton, D.L., Joseph, W.L., Ketcham, A.S., Geelhoed, G.W. and Adkins, P.C.: Surgical resection and adjunctive immunotherapy for selected patients with multiple pulmonary metastases. Ann. Surg., 178: 360-366, 1973.

Cohen, A.M., Ketcham, A.S. and Morton, D.L.: Specific inhibition of sarcoma-specific cellular immunity by sera from patients with growing sarcomas. Int. J. Cancer, 11:273-279, 1973.

Burdick, J.F., Stemple, D. and Wells, S.A., Jr.: Study of the isotopic antiglobulin technique of a cross-reacting murine antibody produced by immunization with a syngeneic SV40 tumor. Int. J. Cancer, 12: 474-484, 1973.

Cohen, A.M., Ketcham, A.S. and Morton, D.L.: Tumor-specific cellular cytotoxicity to human sarcomas: Evidence for a cell-mediated host immune response to a common sarcoma cell-surface antigen. J. Natl. Cancer Inst., 50:585-590, 1973.

Cohen, A.M.: Host immunity to growing sarcomas: Tumor-specific serum inhibition of tumor-specific cellular immunity. Cancer, 31:175-179, 1973.

LeMevel, B.P. and Wells, S.A., Jr.: A microassay for the quantitation of cytotoxic antitumor antibody: Use of ^{125}I-iododeoxyuridine as a tumor cell label. J. Natl. Cancer Inst., 50:585-590, 1973.

Burdick, J.F., Cohen, A.M. and Wells, S.A., Jr.: A simplified isotopic antiglobulin assay: Detection of tumor-cell antigen. J. Natl. Cancer Inst., 50:285-289, 1973.

Burdick, J.F. and Wells, S.A.: Cross-reactivity between cell-surface antigens of different murine carcinogen-induced tumors, demonstrated by a modified isotopic antiglobulin test. JNCI 51:1149-1156, 1973.

LeMevel, B.P., Oldham, R.K., Wells, S.A. and Herberman, R.B.: An evaluation of ^{125}I-iododeoxyuridine as a cellular label for in vitro assays: Kinetics of incorporation and toxicity. JNCI 51:1551-1558, 1973.

Grant, J.P., Ladisch, S. and Wells, S.A.: Immunologic similarities between fetal cell antigens and tumor cell antigens in guinea pigs. Cancer 33:376-383, 1974.

Serial No. NCI-3818
1. Surgery Branch
2. Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Malignancy Associated Cellular and Serum Abnormalities

Previous Serial Number: Same

Principal Investigators: P.B. Chretien, M.D., J.L. Tarpley, M.D., W.J. Catalona, M.D., A.L. Dellon, M.D., and R.H. Yonemoto, M.D.

Other Investigators: C. Potvin, M.D., L.G. Gelrud, M.D., B.F. Cullen, M.D., T.E. Fehniger, B.S., and F.G. Witebsky, M.D.

Cooperating Units: Department of Nuclear Medicine, Clinical Center, NIH; Anesthesiology Department, Clinical Center, NIH; and Laboratory of Pathology, NCI

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description:

Part I. Quantitation of Cellular Immunity in Cancer Patients

A. Correlation of Tumor Histology and Clinical Stage with Cellular and Serum Abnormalities in Cancer Patients

B. Assay of Cellular Immunity in Cancer Patients by Quantitative Dinitrochlorobenzene (DNCB) Contact Sensitivity

C. Thymus-Dependent Lymphocyte Levels as an In Vitro Correlate of Cellular Immunity in Cancer Patients

Part II. Correlation Between Cellular Immunity, Tumor Status, and Prognosis in Patients with Solid Malignancies

A. Correlation of In Vitro Lymphocyte Reactivity and Prognosis after Surgical Treatment of Cancer

B. DNCB Reactivity as a Predictor of Prognosis in Clinically Operable Cancer Patients

C. Comparison of In Vivo and In Vitro Correlates of Cellular Immunity in Tumor-Bearing and Cured Cancer Patients

Part III. Effect of Radiation Therapy on Cellular Immunity

A. Comparison of Cellular Immunity in Cured Patients Treated by Surgery and Radiation Therapy for Laryngeal Carcinoma

B. The Effect of Radiation Therapy for Urologic Cancer on Thymus-Dependent Lymphocyte Levels

Part IV. Cellular Immune Defects in Genitourinary Cancer

A. Abnormalities of DNCB Contact Sensitivity in Patients with Genitourinary Cancer

B. In Vitro Lymphocyte Reactivity to Phytohemagglutinin (PHA) in Patients with Urologic Cancer

C. Thymus-Dependent Lymphocyte Levels in Patients with Bladder and Prostatic Carcinoma

Part V. Correlation of Tumor Status and Cellular Immunity in Patients with Bronchogenic Carcinoma

A. Thymus-Dependent Lymphocyte Levels in Surgically Treated Patients with Bronchogenic and Esophageal Carcinoma

B. Thymus-Dependent Lymphocyte Levels in Patients with Bronchogenic and Esophageal Carcinoma Treated with Radiotherapy

Part VI. The Immunobiology of Skin Cancer

Part VII. Alterations of Serum Ribonuclease Levels in Cancer Patients

A. Correlation of Tumor Histology with Ribonuclease Levels

B. The Relation Between Host Immunocompetence and Serum Ribonuclease Levels in Patients with Urologic Cancers

Part VIII. A New Radioassay for the Quantitation of In Vitro Lymphocyte Reactivity

Part IX. Isolation and Quantitation of Tumor Associated Antigens (TAA)

A. Lymphocyte Reactivity to Tumor Associated Antigens Isolated from Human Tumors

B. Isolation and Characterization of Tumor Associated Antigens from Methylcholanthrene-Induced Tumors in Guinea Pigs

Part X. The Denervated Meissner Corpuscle: A Sequential Histologic Study in the Rhesus Monkey

Project Description:

Part I. Quantitation of Cellular Immunity in Cancer Patients

A. Correlation of Tumor Histology and Clinical Stage with Cellular and Serum Abnormalities in Cancer Patients

Objectives:

To quantitate abnormalities of in vitro lymphocyte reactivity and serum effects on lymphocyte reactivity in cancer patients and determine the incidences of these abnormalities in patients with varied histologic types of tumors and the relationship of tumor stage.

Methods Employed:

Preoperative patients with non-lymphoid tumors of a variety of histologic types were studied. Control data was obtained from healthy volunteers in the same age range as the cancer patients. Volunteers with a history of malignant tumor were excluded. An AB pool, prepared from healthy volunteers, was used as the constant reference source for both cellular and serum reactivity. Purified specimens of mononuclear cells were obtained from the cancer patients and the controls by the exposure of whole blood to carbonyl iron and separation of the cells that phagocytize the iron particles. Cultures containing $5x10^5$ mononuclear cells/ml of 90% RPMI 1640 and 10% AB pooled serum were prepared. Phytohemagglutinin P (PHA) was used in five concentrations to determine the peak response to PHA. Cultures with each dose of PHA and controls without PHA were prepared in triplicate. After a culture interval of 24 hours, tritiated thymidine was added for a three hour labelling period, after which the cultures were terminated. The cultures were processed for scintillation counting and the radioactivity expressed in disintegrations per minute (DPM).

Major Findings:

The cellular reactivity of the cancer patients was arbitrarily defined in terms of the response of normal volunteers: It was normal if it was within 1 SD of the mean for the response of volunteers of the same age group, high if it exceeded the mean by more than 1 SD, and low if it was less than 1 SD of the mean. As a group, significantly more patients with cancer had low reactivity than controls (30% versus 16%). When lymphocyte reactivity was correlated with histology, the highest incidence of low reactivity was seen in patients with squamous carcinomas (45%) and sarcomas (41%). Patients with melanomas and adenocarcinomas as a group had the same lymphocyte reactivity as the control patients. When the patients were divided into three groups according to the stage of tumor growth, there was no correlation between tumor stage and lymphocyte reactivity. Using the AB serum pool as a constant reference, the effect of sera from cancer patients on the reactivity of normal lymphocytes showed that serum from patients with squamous carcinomas and sarcomas significantly inhibited the reactivity of normal lymphocytes. Serum from patients with melanomas and adenocarcinomas did not display this abnormality. Serum and cellular abnormalities coexisted in patients with sarcomas but occurred independently in patients with squamous carcinomas.

Significance to Biomedical Research and Program of the Institute:

A major finding in this study is the association of a greater incidence of low lymphocyte reactivity in patients with tumors of certain histologic types. This information is invaluable in planning immunotherapy regimens that attempt to correct immunological defects. Second, the relationship of low lymphocyte reactivity to development of tumor may be pertinent to the epidemiology and control of cancer. If the low lymphocyte reactivity preceded the tumor in these patients, assays of this type may be useful as a means of screening for cancer-prone individuals and detection of carcinogenic environmental conditions. If low lymphocyte reactivity is the consequence of cancer, whether reversion to higher reactivity occurs with clinical cure should be determined. If it does, assay of lymphocyte reactivity may be a sensitive means of monitoring effects of tumor treatment. The results of this study indicate that only patients with certain histologic types of tumors can be monitored in this manner. The demonstrated relationship of serum suppression factors to tumor histologic type also provides a new, rational basis for the design of immunotherapy treatment protocols based on tumor histology. Failure to demonstrate serum suppressants of lymphocyte reactivity in patients with melanomas and adenocarcinomas may indicate that tumor vaccine treatment for these patients is less likely to have the undesirable effect of suppressing immunity by invoking the production of these serum factors than in patients with epidermoid carcinomas and sarcomas.

Proposed Course:

Studies in progress will determine the nature of the serum inhibitory factor demonstrated and those factors which elicit its production. Control of the action of this factor is of obvious importance in immunologic approach to eradication of malignancy. The effect of immunological adjuvants on the alteration of the suppressed lymphocyte responsiveness is also being studied.

Project Description:

Part I. Quantitation of Cellular Immunity in Cancer Patients

B. Assay of Cellular Immunity in Cancer Patients by Quantitative Dinitrochlorobenzene (DNCB) Contact Sensitivity

Objectives:

To quantitate *in vivo* immunologic responsiveness in patients with non-lymphoid tumors using an assay method that quantitates delayed hypersensitivity (DH) to DNCB.

Methods Employed:

All patients with non-lymphoid tumors admitted to the Surgery Branch for definitive treatment of their malignancies undergo preoperative assay of DH to DNCB. The method employed incorporates the spontaneous flare reaction which occurs to the residual DNCB remaining in the skin after sensitization. A spontaneous flare at the 2000 μg site and the 50 μg site by fourteen days after sensitization is graded 4+. A spontaneous flare at the 2000 μg site only is considered 3+, and a response elicited only after challenge with 50 μg is considered 2+. Reactions that require biopsy to demonstrate are graded 1+. The reactivities of the cancer patients were correlated with tumor histologic type and clinical stage.

Major Findings:

Only 41% of cancer patients developed spontaneous flare reactions to DNCB, an incidence that is significantly less than in controls (96.5%). An additional 29% required challenge to elicit DCH to DNCB, and 30% were anergic. When the patients were grouped according to histologic type of tumor present, all groups had significant abnormal reactivity. The incidence of anergy was highest in patients with squamous carcinomas (43%), less in patients with melanomas (25%) and adenocarcinomas (26%), and not significantly different from controls in patients with sarcomas (12%). Of patients with squamous carcinomas, 28% had impaired positive reactivity which, when combined with the incidence of anergy, made them the most abnormal group. The impaired positive reactivity in patients with melanomas (44%) and adenocarcinomas (26%) further magnifies the abnormality of their expected reactivity. In patients with sarcomas, among whom the proportion of anergy was not significantly different from controls, the 25% incidence of impaired positive reactivity rendered the distribution of their reactivities significantly abnormal when compared with controls. When the patients were divided into two groups according to the extent of tumor determined preoperatively and at operation, and those with localized tumors and those with metastatic tumors, there was no statistically significant difference in the distribution of reactivities in the two groups. When considered by tumor histologic type, patients with sarcomas showed a relationship between impaired reactivity to DNCB and extent of tumor present.

Significance to Biomedical Research and Program of the Institute:

The most important contribution of this assay method is the impaired positive reactivity (2+ and 1+) revealed in allergic cancer patients. These levels of impaired reactivity occurred in a high incidence among patients with tumors of all histologic types studied, thus significantly increasing the proportion in each group with abnormal reactivity. That the incidence of abnormal reactivities was highest in patients with squamous cancer, significantly less in patients with melanoma and adenocarcinoma, and least in those with sarcoma, yet with the exception of sarcoma was not related to clinical extent of tumor, indicates differing host-tumor relationship exists which is determined by the histologic type of tumor present. Therefore, in investigations of cellular immune responses of cancer patients, it is essential to consider tumor histology. This study of cancer patients and the previous study of normal controls demonstrates the method of quantitating DH to DNCB distinguishes at least three levels of reactivity (4+ and 3+, 2+ and 1+, and anergy) which appear to be biologically meaningful.

Proposed Course:

The subsequent clinical course of the patients entering this study will eventually show the relationship of the levels of DH to DNCB demonstrated and prognosis of cancer patients who receive definitive treatment of their tumors. The assay method is also incorporated into an immunotherapy protocol which selects patients on the basis of immunocompetence. The assay will also be used to monitor the effects of the treatment on immune responsiveness.

Project Description:

Part I. Quantitation of Cellular Immunity in Cancer Patients

C. Thymus-Dependent Lymphocyte Levels as an In Vitro Correlate of Cellular Immunity in Cancer Patients

Objectives:

To determine the usefulness of the T cell rosette assay as a correlate of cellular immunity in patients with solid malignancies.

Methods Employed:

The assay of thymus-dependent lymphocyte levels used was developed after extensive studies of techniques of T cell rosette assays that have been previously described. The technique utilized in this study is a modification of previously described techniques with several unique aspects. Lymphocytes separated on Ficoll-Hypaque gradients were added to pooled AB serum and exposed to sheep red blood cells. After incubation, the lymphocytes forming spontaneous rosettes with sheep red blood cells were counted on a Neubauer hemocytometer counter. Both percent T cells and absolute T cells were determined. A total of 240 normal volunteers ranging in age from 20 to 80 were studied to establish normal levels of both percent and absolute T cells. Simultaneously, 94 patients admitted with clinically operable solid tumors (adenocarcinomas, melanomas, sarcomas, and squamous carcinomas of the head and neck and pelvic region) were studied along with 125 patients who were five or more years after definitive treatment and showed no evidence of residual tumor. Statistical analysis of the difference between the groups studied was determined by comparison of the standard error of the difference between means.

Major Findings:

The normal population had a mean lymphocyte level of $1479/\text{mm}^2$ (absolute T cells). The mean percent T cell level of the normal population was 71.5. There was no correlation with age, sex, or race. The preoperative patients with clinically localized tumors had normal levels of circulating lymphocytes. However, both the percent and absolute T cell levels in patients with clinically operable melanomas, sarcomas, and squamous carcinomas were significantly lower than that of the normal population. Patients with adenocarcinomas showed no differences from normals. Patients cured of adenocarcinomas, sarcomas, and melanomas had normal T cell levels (both percent T cell levels and absolute T cell levels). By contrast, patients cured of squamous carcinomas demonstrated persisting low T cell levels similar to their preoperative counterparts. Patients who had received radiation therapy for head and neck cancer had significantly lower T cell levels than non-irradiated patients.

Significance to Biomedical Research and Program of the Institute:

These results show that defects in cellular immunity can be demonstrated in patients with early carcinomas by the spontaneous rosette assay. The increasing evidence for correlation between cellular immunity and the clinical course of cancer patients makes mandatory the development of a rapid, inexpensive, and reproducible method of assaying cellular immunity. The spontaneous rosette assay in this preliminary trial appears to satisfy all of these needs.

Proposed Course:

Further studies of the correlation of thymus-dependent lymphocyte levels as measured by the spontaneous rosette assay developed in this laboratory will be conducted to determine its usefulness as a monitor of conventional and experimental treatment regimens.

Project Description:

Part II. Correlation Between Cellular Immunity, Tumor Status, and Prognosis in Patients with Solid Malignancies

A. Correlation of In Vitro Lymphocyte Reactivity and Prognosis after Surgical Treatment of Cancer

Objectives:

To determine the relationship of *in vitro* lymphocyte reactivity to phytohemagglutinin in patients with non-lymphoid malignancies and prognosis after definitive surgical treatment.

Methods Employed:

The in vitro lymphocyte reactivity was determined in 94 patients who had a variety of non-lymphoid malignancies. In each, the tumor was considered clinically operable and, after determination of the lymphocyte reactivity, definitive surgical removal was attempted. The lymphocytes of 120 volunteers in good health and in the same age range as the cancer patients provided control data for the scoring of the lymphocyte reactivity of the cancer patients. Individuals with a history of malignancy, acute or chronic illness, or who were taking any medication were excluded. In vitro lymphocyte reactivity was determined in autologous serum. Individual leukocyte cultures consisted of $2x10^6$ mononuclear cells in a 4 ml mixture of 80% RPMI 1640 and 20% autologous serum. Phytohemagglutinin P (PHA) was added at five different concentrations ranging from 3.1 to 50 µg of PHA/protein per ml of culture. Triplicate cultures with each dose of PHA and controls without PHA were prepared. After 54 hours incubation, tritiated thymidine was added, and at 72 hours the cultures were terminated. The nuclear protein was precipitated with trichloroacetic acid and the precipitate processed for scintillation counting. The radioactivity of each culture was determined in a Model 303 Packard Tri-Carb scintillation counter. The results were corrected for quench and expressed in disintegrations per minute (DPM).

Major Findings:

To derive an age-correlated range of normal lymphocyte reactivity, the 120 control subjects were divided into five groups of approximately equal age intervals and the mean reactivity ± 1 standard deviation (SD) determined for each group. The lymphocyte reactivities of the control population have a normal distribution, i.e., 84 patients (70%) had lymphocyte reactivity within ± 1 SD of the mean, and 18 patients (15%) each had reactivity greater than and less than 1 SD of the mean. The highest lymphocyte reactivity occurred in the youngest individual studied. From a peak in the second decade, reactivity declined over the next four decades, then was relatively constant through the last decade studied.

Three years after completion of the determination of lymphocyte reactivity of the cancer patients, the medical records of the patients were reviewed. Follow-up was complete on all patients. The patients were classified either as (1) clinically cured of tumor at the time of the review (34 patients), (2) as inoperable (29 patients), or (3) as having recurrent tumor - either at the site of operation or in the form of distant metastases - after tumor resection that was considered complete (31 patients). The lymphocyte reactivity of each patient was arbitrarily defined in relation to the reactivity of the control population. The reactivity within ± 1 SD of the mean age-correlated control reactivity was classified as normal, reactivity greater than 1 SD of the mean control reactivity was classified as high, and reactivity less than 1 SD of the mean control reactivity was classified as low. The distribution of the lymphocyte reactivity of the cancer patients was significantly lower than that of the controls. The difference is due primarily to the percent of cancer patients with low lymphocyte reactivity (45% compared with 15% of controls). Significantly fewer cured cancer patients, however, had low lymphocyte reactivity than did inoperable patients or patients with recurrent tumor. There was a significantly greater percent of cured patients with inoperable or recurrent tumors (7%).

Significance to Biomedical Research and Program of the Institute:

The relationship shown in this study of in vitro lymphocyte reactivity to extent of tumor growth and prognosis after surgical treatment shows that this test assays a clinically significant aspect of the host-tumor relationship. The studies which demonstrate a correlation between the delayed hypersensitivity response to dinitrochlorobenzene and prognosis in cancer patients and those which show that the DH response to DNCB parallels in vitro lymphocyte reactivity when considered together suggest that in vitro lymphocyte reactivity provides similar insight into the prognosis of cancer patients as DH to DNCB. These two tests combined, therefore, may have increased value as a means of predicting the course of cancer patients who receive definitive treatment of their malignancies.

Proposed Course:

Continued improvement in this assay system to provide more quantitation of in vitro lymphocyte reactivity and extension of the assay to include patients clinically cured of tumor, and continued assay of patients studied preoperatively will further determine the relationship of in vitro reactivity to susceptibility to cancer, effect of cancer treatment, and the feasibility of adjunctive therapy which operates through alteration of immunologic responsiveness.

Project Description:

Part II. Correlation Between Cellular Immunity, Tumor Status, and Prognosis in Patients with Solid Malignancies

B. DNCB Reactivity as a Predictor of Prognosis in Clinically Operable Cancer Patients

Objectives:

To evaluate usefulness of DNCB to the clinician as a diagnostic and prognostic indicator.

Methods Employed:

Patients admitted to the Surgery Branch of the National Cancer Institute over the preceding three years have had DNCB skin testing by a quantitative method employing a 2000 μg and 50 μg sensitization and a challenge of 50 μg if there was no spontaneous flare after 14 days. The clinical courses of 268 patients admitted with clinically operable tumors and tested for reactivity to DNCB were evaluated.

Major Findings:

DNCB responsiveness varies with histology. There is an increased incidence of anergy in patients with squamous carcinomas and with sarcomas. There is an increased incidence of impaired responses and anergic responses in patients with adenocarcinomas. There were no significant differences between the distribution of responses in patients with melanoma and the normal population. DNCB response did not correlate with a short-term (mean 18 months) prognosis after definitive surgical treatment for cancer. DNCB response did not correlate with operability. A number of factors influence and depress DNCB responsiveness, the major two being prior irradiation and intercurrent anesthesia or operation. DNCB responses in patients with melanoma were not influenced by the stage of disease in clinically operable patients. There were no differences between the subtypes of sarcoma in their distribution of responses to DNCB.

Significance to Biomedical Research and Program of the Institute:

Adequate immunotherapy requires monitoring alterations in immune reactivity. One avenue of research converging on this point has been the use of various primary antigens, delayed cutaneous hypersensitivity assays of which one of the most principally investigated has been DNCB. Our findings are that DNCB responses must consider histology and other factors, including irradiation and intercurrent anesthesia and/or operation. DNCB does not appear to offer a prognostic guide, since patients who proved to be inoperable were not distinguished from patients who were operable. Likewise, the lack of correlation of responsiveness or lack of responsiveness with prognosis mitigated against the modification of therapy on the basis of DNCB response.

Proposed Course:

DNCB responsiveness does appear to be a gross measure of immune competence. It does not help predict operability or prognosis. Changes in an individual patient's response to DNCB may well correlate with clinical manifestations of host immune competence. For this reason, we propose in the future to test DNCB responsiveness approximately one month after surgery after the gross tumor burden has been removed to establish the baseline at this point and to periodically rechallenge the patients with DNCB to test for any conversions either from negative to positive or positive to negative which might serve as a monitor of the current status of the tumor-host relationship.

Project Description:

Part II. Correlation Between Cellular Immunity, Tumor Status, and Prognosis in Patients with Solid Malignancies

C. Comparison of In Vivo and In Vitro Correlates of Cellular Immunity in Tumor-Bearing and Cured Cancer Patients

Objectives:

To quantitate cellular immunity in cured cancer patients utilizing in vitro reactivity to phytohemagglutinin (PHA) and in vivo contact sensitivity to dinitrochlorobenzene (DNCB) and to compare the reactivity of these patients with that of preoperative cancer patients having the same histologic types of tumors.

Methods Employed:

Over 100 patients who had been treated for histologically proved malignancies at the Surgery Branch were studied. The patients were divided into five groups according to the histologic type of tumor excised - head and neck squamous cancer, pelvic squamous cancer, sarcomas, melanomas, and adenocarcinomas. All patients were in good general health at the time of the study, and each had undergone a physical examination within three months of the study and were considered clinically cured of tumor at the time. The mean interval since treatment of their primary tumor was eight years and the range five to fourteen years.

Lymphocyte reactivity to PHA and skin contact sensitivity to DNCB were determined by the methods described in the preceding parts of this section. The data derived from these patients were compared with that obtained from study of the preoperative patients and the normal volunteers who were studied during the same time interval. The data derived from these patients have been described in the previous parts of this section.

Major Findings:

The lymphocyte reactivity of patients cured of cancer correlated with tumor histology. Among patients previously treated for squamous carcinomas of the head and neck region and female pelvic organs, there was an increased incidence of patients with low reactivity similar to that found in preoperative patients. Among patients previously treated for sarcomas, melanomas, and adenocarcinomas, the incidence of high reactivity exceeded that of the normal population. Comparison of the high reactivity of cured sarcoma and melanoma patients with the low reactivity of their preoperative counterparts showed a statistical difference of $p < .05$ for the sarcoma patient groups and $p < .01$ for the melanoma patient groups. The effect of the serum of the cancer patients on the reactivity of normal lymphocytes also correlated with tumor histology and the findings were similar in both preoperative and cured patients. In both groups serum from patients with squamous carcinomas and sarcomas significantly inhibited normal lymphocyte reactivity, while serum from patients with melanomas and adenocarcinomas did not have an inhibitory effect.

The distribution of DNCB reactivities among patients cured of cancer also correlated with tumor histology. Among patients with sarcomas, melanomas, and adenocarcinomas, the distribution of reactivities did not differ from that of the normal population. By contrast, patients cured of squamous carcinomas of the head and neck region and female pelvic organs continued to show the abnormal reactivity demonstrated in preoperative patients.

Significance to Biomedical Research and Program of the Institute:

The findings of normal cellular immunity among patients with sarcomas, melanomas, and adenocarcinomas suggest that patients previously treated for tumors of these histologic types who have impaired cellular immunity may be at high risk for having residual tumor. If greater effectiveness of radiotherapy, chemotherapy, and immunotherapy is achieved in patients with relatively small tumor burdens, an important application of the assays used may be for the identification of patients who have residual tumor before it is clinically manifest, to allow earlier institution of therapy. The persistence of immune defects in cured squamous cancer patients provokes questions concerning the role of genetic and environmental factors in the genesis of these tumors. For instance, are these patients from a cohort of the population with preexisting and perhaps genetically determined abnormalities of thymus-dependent lymphocyte function? Do then the preexisting immune defects predispose to cancer by increasing the susceptibility to environmental carcinogens? Is a preexisting immune impairment a prerequisite for induction of cancer by agents which have been linked to squamous carcinogenesis? The high incidence of impaired cellular immunity in squamous cancer patients may also serve as a rational basis for selection of radiotherapy or surgery for lesions which are appropriately treated by either modality. In view of the evidence for an immunosuppressive effect of immunotherapy, patients with impaired immunity might be selected for surgical treatment, while those with normal reactivity may be more favorable candidates for radiotherapy.

Proposed Course:

Serial assays of cellular immunity in patients treated for sarcomas, melanomas, and adenocarcinomas are in progress to determine whether those patients with low reactivity revert to higher reactivity after tumor removal or, conversely, if patients with low reactivity fail to be cured despite definitive surgery. To further elucidate the factor accounting for the persisting immune defects in patients cured of squamous carcinomas, investigation of the genetic profiles of patients with these histologic types of tumors by determination of HL-A genotypes is in progress.

Project Description:

Part III. Effect of Radiation Therapy on Cellular Immunity

A. Comparison of Cellular Immunity in Cured Patients Treated by Surgery and Radiation Therapy for Laryngeal Carcinoma

Objectives:

This study was designed to determine the effects of local irradiation for cancer to an area not involving the thymus, major portions of bone marrow, or other portals encompassing a major portion of the lymphoid system. These studies were conducted in patients with sufficient disease-free intervals after treatment to consider them cured to isolate the effects of irradiation.

Methods Employed:

Patients were selected who had been treated for carcinoma of the laryngopharynx at least four years previously. Forty-four normal adult volunteers were selected as controls of the in vitro assays of lymphocyte reactivities, serum effects on lymphocyte reactivity, and assays of immunoglobulin levels. A total of 187 healthy volunteers served as controls in the thymus-dependent lymphocyte (T cell) assay. Patients and volunteers alike with histories of chronic diseases, medication, acute illnesses, or recent immunizations were excluded. Controls and cancer patients were studied concurrently. Four assays were employed: (1) Lymphocyte reactivity to phytohemagglutinin (PHA). Heparinized blood was sedimented with methyl cellulose to obtain white cell suspensions which were exposed to carbonyl iron to obtain a mononuclear cell suspension. Individual cultures of $5x10^5$ lymphocytes/ml in 90% RPMI 1640 and 10% pooled AB serum were employed. PHA was added in varying concentrations. The mixture was incubated for 54 hours at 37°C. and then tritiated thymidine (30mCi/ml of culture) was added. Incubation was continued for 3 hours, at which time cells were frozen. Nuclear protein was precipitated, washed, solubilized, and finally counted in a scintillation counter. The reactivity of the lymphocyte population was defined as the highest reactivity recorded regardless of PHA concentration at which it occurred minus the reactivity of cultures without PHA. (2) The T cell assay. Blood was layered on a Ficoll-Hypaque gradient; mononuclear cells formed a white disc at the interface. The mononuclear cells were concentrated, washed, and added to washed sheep erythrocytes in RPMI 1640 and human AB serum free of complement. After absorption this mixture was incubated, centrifuged, allowed to stand, and finally counted in a hemocytometer. By convention, any lymphocyte with three or more adherent sheep erythrocytes was considered a T lymphocyte. The corresponding total and differential leukocyte counts were obtained from which the total lymphocyte count was calculated. The absolute T cell count was the product of the percent T cells and the total lymphocyte count. (3) Quantitation of serum immunoglobulin levels. The automated nephelometric method was employed. Specific precipitating antisera was incubated with the test sera. The resulting antigen-antibody complexes scatter light in direct proportion to the concentration of antigen present. (4) Serum effect on in vitro lymphocyte reactivity. The effect of test sera on reactivity of normal lymphocytes to PHA was compared to the reactivity of the same lymphocytes and the pooled AB serum. The quotient of the former divided by the latter is termed the lymphocyte reactivity index.

Major Findings:

In patients who were 4-23 years, mean 8.4 years, after surgical treatment alone for laryngopharyngeal cancer neither cellular nor humoral immunity differed from that of 44 controls. In contrast, 14 patients who had been irradiated and subsequently cured for 4-15 years, mean 9.0 years, prior to evaluation, displayed significantly impaired cellular immune competence compared to normal and also when compared to patients treated by surgery alone. Except for higher IgA levels in the irradiated patients, no differences in the two parameters of humoral immunity, immunoglobulin level, and serum effect on lymphocyte reactivity were found.

Significance to Biomedical Research and Program of the Institute:

This study documents that patients with sufficiently long disease-free intervals to consider them clinically cured of cancer who received local radiation therapy not involving the thymus, large areas of red bone marrow such as the pelvis, or other lymphoid organs displayed persisting suppression of cellular immune competence. This study demonstrates that the effect of irradiation can be systemic and prolonged. These findings of prolonged impairment of systemic cellular immunity may have relevance to the finding of an increased incidence of systemic metastases in patients treated with adjunctive irradiation for carcinoma of the breast and head and neck region. The findings of this study may also have relevance to the selection of therapeutic alternatives in the treatment of cancer.

Proposed Course:

An attempt will be made to initiate a treatment protocol that utilizes the initial immune response of the tumor-bearing head and neck patient as a guide for the selection of therapeutic alternatives. Patients with normal cellular immune competence would be randomized to either surgical treatment or radiation therapy. Likewise, patients with impaired cellular immune competence would be randomized to the two modalities. At attempt will be made to determine whether patients with impaired cellular immunity are best treated by surgery since the immunosuppressive effects of radiation therapy in this group when combined with the patient's preexisting impaired immunity may result in lower survival rates than would be obtained with surgical treatment. An additional study proposed is the determination of the effects of immune stimulants, such as BCG, in patients with impaired cellular immunity when administered during the course of radiation therapy.

Project Description:

Part III. Effect of Radiation Therapy on Cellular Immunity

B. The Effect of Radiation Therapy for Urologic Cancer on Thymus-Dependent Lymphocyte Levels

Objectives:

To evaluate T rosette forming lymphocytes in patients who had received radiation therapy for urologic tumors and compare these levels to their counterparts with similar stages of disease.

Methods Employed:

The T rosette assay as described in previous sections was employed. Basically, lymphocytes were separated from peripheral blood on a Ficoll-Hypaque gradient and were incubated in the presence of AB positive serum and sheep red blood cells for 15 minutes at 37°C. The preparations were then centrifuged and the rosette preparations were resuspended and the percentages of T lymphocytes were determined. Corresponding total and differential leukocyte counts were obtained from which the total T cell levels were calculated.

Major Findings:

This study of T rosette lymphocytes in 50 patients with urologic tumors, of whom 18 were irradiated and 32 were not, revealed that local radiation therapy for urologic cancer not including the thymus produces more than a 50% reduction in the total circulating lymphocytes with a selective loss from the T cell compartment (68%, persisting for more than one year). The results indicate that the systemic immunosuppressive consequences of local radiation therapy may increase the prospects of implantation and colonial outgrowth of circulating tumor cells which escape the radiation fields before the initiation of therapy. The indications for adjunctive radiotherapy for urologic cancer should be weighed in the light of these findings.

Significance to Biomedical Research and Program of the Institute:

All cancer therapy is potentially immunosuppressive. The accumulating evidence for the immunosuppressive effect of radiation therapy indicates the need for studies which determine whether the patients who fail to respond to radiotherapy consist of those who as a group have a higher incidence of impaired immune responsiveness. If this is so, these patients may benefit from adjunctive immune stimulants during the course of radiotherapy.

Proposed Course:

As discussed in the preceding part of this project (Part III A), attempts will be made to incorporate this data into clinical trials to determine whether patients presenting with genitourinary cancers who are candidates for radiotherapy and who have impaired cellular immunity would have higher cure rates if immune stimulants such as BCG were administered prior to and during the course of radiotherapy.

Project Description:

Part IV. Cellular Immune Defects in Genitourinary Cancer

A. Abnormalities of DNCB Contact Sensitivity in Patients with Genitourinary Cancer

Objectives:

To evaluate the relationship between cell-mediated immunocompetence and tumor stage and tumor histological type in genitourinary cancer.

Methods Employed:

Dinitrochlorobenzene (DNCB) contact sensitization was employed. Doses of 2000 μg and 50 μg were applied to areas 2 cm in diameter on the upper arm and forearm respectively. These sites were observed 24 hours later for an irritative reaction to test the integrity of the inflammatory response and 2 weeks later to assess the specific cell-mediated immune response to DNCB. The basis for quantitating reactivity incorporates the spontaneous flare reaction which occurs to the residual DNCB remaining in the skin 1 to 2 weeks after application. A spontaneous flare reaction at the 2000 μg and 50 μg dose sites is scored 4+. A spontaneous flare only at the 2000 μg dose site is scored 3+. In the absence of a spontaneous flare, a challenge dose of 50 μg is applied to the forearm and if an unequivocal delayed cutaneous hypersensitivity reaction occurs to this challenge dose the reaction is scored 2+. In rare circumstances an equivocal reaction will occur to this dose; this reaction is biopsied with a skin punch and examined for the histologic features of delayed cutaneous hypersensitivity. If these features are present the reaction is scored 1+; if they are not or if no reaction occurs to the challenge does the test is scored as anergic.

Major Findings:

DNCB reactivity from 143 healthy volunteers ranging in age from 20 to 80 years provided age-correlated control data for normal DNCB reactivity. Sixty-four patients were studied, all of which were bearing tumors at the time of skin testing. Twenty-three patients had transitional carcinomas, including one with renal pelvic tumor and 22 with bladder tumors. Eight renal cell carcinomas were studied, 25 patients with prostatic adenocarcinomas were studied, and 6 patients with germinal testis tumors were studied. Subnormal immunologic responses were demonstrated by the DNCB skin test in 57% of patients with transitional carcinoma, 52% of patients with prostatic carcinoma, 50% of patients with renal cell carcinoma, 50% of patients with penile carcinoma, and 17% of patients with testis tumors.

The incidence of impaired immunocompetence correlated with the clinical extent of tumor in patients with transitional carcinoma: 100% of patients with metastatic disease had impaired DNCB reactivity and 38% with localized disease had impaired DNCB reactivity. A trend toward correlation was also observed in patients with renal cell carcinoma: 60% with metastatic disease had impaired DNCB reactivity while 33% with localized disease had impaired immunocompetence. No significant correlation was demonstrated between host immunocompetence and tumor stage with prostatic carcinoma: 47% with metastatic disease and 60% with localized disease had impaired immunocompetence. No correlation was demonstrated among patients with testicular carcinoma or with penile carcinoma.

However, the numbers of patients studied in the latter two groups were small. Among patients with transitional carcinoma, significant correlations were demonstrated between impaired host immunocompetence and tumor stage, tumor grade, and the presence or absence of vascular or lymphatic permeation. All patients with successfully treated transitional carcinomas had normal DNCB reactivity.

Significance to Biomedical Research and Program of the Institute:

The demonstration of a correlation between tumor stage, tumor grade, and the presence or absence of vascular or lymphatic permeation in patients with transitional cell carcinoma is the first such demonstration between host immunocompetence and tumor extent in patients with any solid neoplasms. The demonstration of normal immunocompetence in patients who were free of cancer suggests that host defenses are important in determining the prognosis of a patient with transitional carcinoma who has received definitive surgical therapy.

Proposed Course:

These studies will be continued and expanded to the evaluation of cellular immunity during the course of treatment and after treatment. Correlations will be made with the severity of immune depression before and during treatment and cure rates achieved. This information will provide a rational basis for the design of treatment regimens which preserve immune competence.

Project Description:

Part IV. Cellular Immune Defects in Genitourinary Cancer

B. In Vitro Lymphocyte Reactivity to Phytohemagglutinin (PHA) in Patients with Urologic Cancer

Objectives:

To determine the relationship between lymphocyte responsiveness to phytohemagglutinin (PHA) in patients with urologic cancer and tumor type, tumor stage, and the effects of previous irradiation.

Methods Employed:

Peripheral blood was obtained by venipuncture from 53 patients with urologic cancer and lymphocytes were purified by sedimentation in 1% methyl cellulose after exposure to carbonyl iron. Mononuclear cells were washed three times in RPMI 1640 tissue culture media and placed in tissue culture concentrations of 500,000 lymphocytes/cc of culture media. Ten percent human AB serum was used to supplement all cultures. Lymphocyte cultures were stimulated with serial dilutions of PHA in doses of 25, 12.5, 6.25, and 3.125 μg of PHA protein per culture. Unstimulated control cultures were all included in which no

PHA was added. All samples were done in triplicate. Lymphocytes were incubated for 54 hours in 95% air, 5% CO_2 and were pulsed with tritiated thymidine for three hours. After this, cultures were terminated by quick freezing in liquid nitrogen, and the acid precipitatable nucleoprotein was counted for incorporation of radioactive labeled thymidine. Activity was expressed in disintegrations per minute. Normal reactivity had been determined by the concurrent study of age-matched healthy controls cultured under identical conditions. Furthermore, the effect of serum from patients with urologic cancer on the responsiveness of normal allogeneic lymphocytes was determined as has been described previously. Briefly, allogeneic lymphocytes from healthy volunteers were cultured in the test serum from either a cancer patient or normal control and compared to the reactivity of the same lymphocyte population in an AB positive serum pool.

Major Findings:

The lymphocyte reactivity of patients with metastatic bladder transitional carcinomas was significantly depressed; however, the mean lymphocyte reactivity of patients with localized transitional carcinoma did not differ significantly from controls. Among patients with prostatic carcinoma, lymphocyte reactivity was significantly depressed in both patients with localized disease and those with metastases present. A strong trend toward depressed lymphocyte reactivity was observed in patients with metastatic renal cell carcinoma, although no statistical significance could be achieved because of the small number of patients studied. Patients with localized renal cell carcinoma had lymphocyte reactivity which did not differ from controls. Lymphocyte reactivity in patients with testis tumors was also not different from controls. However, lymphocyte reactivity from patients who had received radiation therapy for any urologic tumor was significantly suppressed when compared with controls.

Significance to Biomedical Research and Program of the Institute:

These studies which show a correlation with tumor stage and cellular immunity and tumor extent in patients with genitourinary cancer contribute a potential objective parameter by which patients may be selected for alternative treatment regimens. The emerging evidence for the importance of maintenance of immune competence as evidenced by the increased incidence of malignancies in patients on immunosuppressive regimens gives importance to the evaluation of the effects of immune stimulants during the administration of immunosuppressive cancer regimens in patients who present with impaired cellular immunity.

Proposed Course:

These studies will be continued and expanded to the evaluation of cellular immunity during the course of treatment and after treatment. Correlations will be made with the severity of immune suppression before and during treatment and cure rates achieved. This information will provide a rational basis for the design of treatment regimens which preserve immune competence.

Project Description:

Part IV. Cellular Immune Defects in Genitourinary Cancer

C. Thymus-Dependent Lymphocyte Levels in Patients with Bladder and Prostatic Carcinoma

Objectives:

To quantitate levels of T rosette forming lymphocytes in patients with prostatic and bladder carcinoma.

Methods Employed:

Forty-six patients with proven malignancies were studied: 25 had prostatic carcinoma and 21 had bladder carcinoma. Tumors were staged according to the systems proposed by the Veterans Administration Urological Research Group and the International Union Against Cancer. All patients were either preoperative or at least five days postoperative. No patient had received previous irradiation or chemotherapy. The technique used in the T rosette assay has been described in preceding portions of this report. Briefly, lymphocytes were separated from peripheral blood on a Ficoll-Hypaque gradient, were washed three times and incubated for 15 minutes at 37°C. with a 1% solution of sheep erythrocytes and human AB positive serum which had been absorbed against the same erythrocytes after heat inactivation at 56°C. for 30 minutes. The preparations were then centrifuged and incubated at 4°C. for at least one hour before counting in a hemocytometer.

Major Findings:

Both the percentage of peripheral blood T rosette forming lymphocytes and the absolute number of T rosette forming lymphocytes were impaired in patients with both prostatic and bladder carcinoma. However, when patients were categorized according to the extent of tumor present, patients with localized bladder carcinoma had normal absolute T lymphocyte counts while patients with metastatic bladder carcinoma had significantly low T lymphocyte counts. In contrast, among patients with prostatic carcinoma, no significant difference in the percentage of T lymphocytes in the peripheral blood or the absolute T lymphocyte levels in the peripheral blood was demonstrated. In general these findings were in concert with parallel studies using phytohemagglutin and DNCB reported in Parts A and B and indicate that the impaired cellular immune responsiveness observed in patients with prostatic and bladder carcinoma is in part due to a reduced number of T lymphocytes in the peripheral blood. Furthermore, the emerging pattern of impaired immunocompetence being more prevalent among patients with advanced bladder carcinoma than among their counterparts with localized disease suggests that prognosis may be determined by host immunocompetence in patients with transitional carcinomas. However, the absence of a correlation of patients with prostatic carcinoma suggests that perhaps the induction and progress of this neoplasm is mediated through an entirely different mechanism than that observed in patients with transitional carcinoma.

Significance to Biomedical Research and Program of the Institute:

These studies which show a correlation with tumor stage and cellular immunity and tumor extent in patients with genitourinary cancer contribute a potential objective parameter by which patients may be selected for alternative treatment regimens. The emerging evidence for the importance of maintenance of immune competence as evidenced by the increased incidence of malignancies in patients on immunosuppressive regimens gives importance to the evaluation of the effects of immune stimulants during the administration of immunosuppressive cancer regimens in patients who present with impaired cellular immunity.

Proposed Course:

These studies will be continued and expanded to the evaluation of cellular immunity during the course of treatment and after treatment. Correlations will be made with the severity of immune suppression before and during treatment and cure rates achieved. This information will provide a rational basis for the design of treatment regimens which preserve immune competence.

Project Description:

Part V. Correlation of Tumor Status and Cellular Immunity in Patients with Bronchogenic Carcinoma

A. Thymus-Dependent Lymphocyte Levels in Surgically Treated Patients with Bronchogenic and Esophageal Carcinoma

Objectives:

To determine the correlations between thymus-dependent lymphocyte levels (T cell levels) with the clinical tumor status of the patient: (a) tumor-bearing, pretreatment patients of various tumor stages, (b) with respect to tumor histology, (c) in surgically cured patients, and (d) pretreatment levels with posttreatment clinical course.

Methods Employed:

Thymus-dependent lymphocyte levels were determined by the formation of spontaneous rosettes between human peripheral blood lymphocytes and sheep red blood cells. Fifteen ml of the patient's heparinized blood was layered on a Ficoll-Hypaque gradient to obtain lymphocyte separation. The lymphocytes then were washed to remove serum factors and placed with sheep red blood cells in pooled human serum to form the spontaneous rosettes. These then were counted and percent T cells in the peripheral blood obtained. This percentage was multiplied by the peripheral white blood cell count and the percentage lymphocytes in the white blood count differential to obtain the peripheral blood

T cell levels. Two hundred patients with lung cancer were studied. Patients were excluded from this study if they had had previous immunosuppressive therapy which included either radiotherapy or cytotoxic or steroid type drugs or general anesthesia during the preceding week. Patients were also excluded if they had received any immunostimulation type of therapy such as blood transfusions within the previous three weeks. Patients also were excluded if the tissue obtained for their diagnosis was not sufficient to permit a histologic diagnosis of bronchogenic carcinoma. Patients with non-bronchogenic pulmonary carcinomas were also excluded. The remaining 112 patients were grouped according to tumor histology: squamous cell adenocarcinoma, oat cell, or undifferentiated carcinoma; and within each histologic grouping they were staged according to whether the tumor extent was localized, regional, or metastatic. Cured patients were those who had only previous surgical treatment. In those patients in whom tumors could be resected, T cell levels were obtained monthly during the postoperative course. Thirty patients with esophageal carcinoma were also included in this study and subsequent groupings were utilized where applicable.

Major Findings:

For patients with squamous cell carcinoma, oat cell carcinoma, and undifferentiated carcinoma of the bronchus the pretreatment T cell levels were all significantly lower than normal, and the T cell levels continued to decrease with increasing extent of the tumor. For patients with adenocarcinoma the T cell levels all were significantly lower than normal but there was not a significant decrease of T cell levels with increasing extent of tumor. Patients cured of squamous cell carcinoma of the lung had persistent depression of T cell levels which were significantly lower than normal, whereas those patients cured of adenocarcinoma of the lung had T cell levels that were normal. Pretreatment T cell levels that were less than 500 were related to an extremely poor prognosis, with 90% of the patients being dead from metastatic disease or alive with disease within an average of four months. Patients followed sequentially during the postoperative period who developed metastatic disease had a progressive sequential decrease in their T cell levels, which averaged 40% prior to the clinical development of metastatic disease. Patients with both adenocarcinoma and squamous carcinoma who remained clinically free of disease in the postoperative period demonstrated an increase in their T cell levels. Those patients who had T cell levels which remained constant or decreased slowly during the postoperative period developed metastatic disease; however, this did not occur for at least six months. Patients with esophageal carcinoma demonstrated a corresponding low T cell level, and this T cell level corresponded with subsequent clinical postoperative course in a manner identical to those patients with bronchogenic carcinoma.

Significance to Biomedical Research and Program of the Institute:

Demonstration that T cell levels are low in patients with bronchogenic carcinoma and that these levels decrease with increasing extent of disease and have variation depending on tumor histology demonstrates the importance of monitoring cell-mediated immunity in the host-tumor relationship and in the management of these patients. It should prove possible to determine whether those people who appear by all clinical tests to have localized resectable disease have T cell levels that are related to more advanced regional or metastatic disease so that patients in addition to their surgical resection may be entered into adjuvant surgical programs. In addition, people during the postoperative period who appear to have had their tumor removed at surgery but whose T cell monitoring suggests successive decreases in T cell levels could be entered into surgical adjuvant programs several months prior to the development of clinically metastatic disease.

Proposed Course:

This first correlation of postoperative course with a parameter of cellular immunity in individual cancer patients provides a basis for the determination of those patients who will develop recurrent tumor prior to the gross manifestations of recurrence. The assay thus provides an early warning system which should be utilized as an indication for the institution of adjunctive therapy, such as chemotherapy or immunotherapy.

Project Description:

Part V. Correlation of Tumor Status and Cellular Immunity in Patients with Bronchogenic Carcinoma

B. Thymus-Dependent Lymphocyte Levels in Patients with Bronchogenic and Esophageal Carcinoma Treated with Radiotherapy

Objectives:

To determine correlations between thymus-dependent lymphocyte levels and the clinical response of patients with bronchogenic and esophageal carcinoma to radiotherapy and to determine the effect of radiotherapy upon T cell levels in these patients.

Methods Employed:

Thymus-dependent lymphocyte levels were determined by the formation of spontaneous rosettes between human peripheral blood lymphocytes and sheep red blood cells. Fifteen ml of the patient's heparinized blood was layered on a Ficoll-Hypaque gradient and the lymphocytes obtained. The lymphocytes then were added to sheep red blood cells and pooled human AB serum to form these spontaneous rosettes. Rosettes then were counted under the microscope and the percent T cells obtained. This percentage was then multiplied by the patient's white blood cell count and the percent lymphocytes in the white count differential to obtain the T cell level. Seventy patients with bronchogenic carcinoma and 20 patients with esophageal carcinoma receiving radiotherapy either as the primary treatment or as an adjuvant to surgical resection of the tumors were included in this study. Patients were excluded who had previous cytotoxic drugs or steroid therapy or who had previous blood transfusions or in whom there was insufficient tissue obtained to permit an accurate histologic diagnosis of the tumor. Patients were studied before, during, and at monthly intervals following radiotherapy.

Major Findings:

In all patients receiving radiotherapy the T cell levels fell progressively throughout the course of radiotherapy. An average of 70% decrease in T cells was found for a cumulative dose of 5000 rads to the mediastinum. Patients whose T cell levels fell below 400 prior to receiving a cumulative dose of 4000 rads developed metastatic disease within 2 months. Patients at 1 month following radiotherapy who were to remain free of disease demonstrated an increase in their T cell levels. Patients at 1 month following radiotherapy who were to not respond to the radiotherapy, and who were to have either persisting tumor or development of metastatic disease demonstrated either a continuing decline in T cell levels following radiotherapy or a constant level of T cells during radiotherapy, or occasionally a patient would have a level rise to 600 T cells and then decline. Patients evaluated at later intervals following radiotherapy remained free of disease during times at which their T cell levels were increasing or remained constant. However, drops in T cell levels heralded by 2 to 3 months the onset of clinically evident metastatic disease. There was no difference between the findings for patients with bronchogenic or esophageal carcinoma.

Significance to Biomedical Research and Program of the Institute:

The demonstration that decreases in sequentially monitored T cell levels corresponds to the subsequent development of clinically evident metastatic disease suggests that there are several points during the treatment of bronchogenic and esophageal carcinoma with radiotherapy at which patients could be entered into adjuvant chemotherapeutic programs or protocols. For instance, if during the course of radiotherapy the T cell levels decreased at a rate greater than that expected for patients receiving radiotherapy alone, i.e., if their T cell levels fall below 400 prior to receiving a cumulative dose of 4000 rads, these people could either during or immediately subsequent to radiotherapy receive adjuvant treatment. If at 1 month following therapy the patient's T cell levels fail to increase to levels greater than 600 the patient could be entered into an adjuvant program. If during longer postradiotherapy intervals monitoring suggested decreases in the T cell levels on more than one determination, the patient could be entered into adjuvant programs.

Proposed Course:

These findings should be in corporated into treatment regimens for patients with bronchogenic and esophageal carcinoma. Adjunctive immunotherapy and chemotherapy may be of potential benefit to patients whose T cell levels decline below 400 prior to the completion of radiotherapy and to patients whose T cell levels fail to rebound to a higher level at 1 month after completion of radiotherapy.

Project Description:

Part VI. The Immunobiology of Skin Cancer

Objectives:

The immunobiology of non-melanoma type skin cancer was investigated with a multiplicity of approaches which incorporated (a) determination of cellular immunity by thymus-dependent lymphocyte levels, (b) evaluation of genetic factors by histocompatibility (HL-A) typing, and (c) host-tumor relationship *in vivo* through estimating degree of lymphocytic infiltration of the tumor.

Methods Employed:

(a) The thymus-dependent lymphocyte levels in patients with squamous carcinoma, basal cell carcinoma, Bowen's disease, and xeroderma pigmentosa were determined by the formation of spontaneous rosette formation between sheep red blood cells and the patient's peripheral blood lymphocytes. The technique used was that of placing heparinized peripheral blood from the patient onto a Ficoll-Hypaque gradient, then taking the separated lymphocytes and adding to them sheep red blood cells in pooled human serum to form spontaneous rosettes. These then were counted and the percent spontaneous rosettes or T cells determined. This percent was multiplied by the patient's peripheral white blood cell count and by the percent of lymphocytes in the peripheral blood to achieve the T cell level for the patient. These values were compared to 200 non-cancer control patients. (b) Fifteen ml of peripheral blood was placed into a Terasaki transport bag and the histocompatibility type of the patient was subsequently determined through the microtiter assay of Terasaki. Twenty-one HL-A specificities were tested and the antigen frequencies compared to a panel of 200 non-cancer patients obtained through hospital volunteers and the hospital Blood Bank. (c) Resected tumor specimens in the NIH Pathology Department were examined histologically in those patients in whom T cell levels were also obtained. The degree of lymphocytic infiltration of the tumor was estimated from absent to minimal to marked, and this estimate was then correlated with the size of the tumor and the T cell level.

Major Findings:

T cell levels were found to be depressed to a level significantly lower than normal in all patients bearing skin cancers. The levels, however, were significantly lower in those patients with large tumors, those greater than 2 cm, than in those with small tumors. Patients surgically cured of their skin cancer, those who had had small tumors, had T cell levels that were in the normal range. Those patients cured of large tumors had persistently low T cell levels. There was no difference between patients who had had basal cell or squamous cell carcinomas. Patients surgically treated for Bowen's disease had as a group a T cell level significantly lower than normal. When analyzed by T cell levels of the individual patients compared to those in the normal population, a significant increase in the number of patients with very low (greater than 2 standard deviations below normal) T cell levels was found. Patients with xeroderma pigmentosa had normal T cell levels when they were free of their tumors. When skin cancer patients were analyzed as a group, there was an increase in HL-A frequencies 12 and W17. This increase did not remain significant when corrected by the number of specificities tested for. However, when patients with Celtic backgrounds were examined separately an increase that remained significant even when corrected for the number of specificities was found for HL-A frequencies 12 and W17. HL-A frequencies 1 and 8 were increased in this group significantly from the control population, but the values did not remain significant when corrected. When the HL-A types for those patients with large tumors and those with low T cell levels (less than 1000) were examined, there was an increase in HL-A frequencies 1 and 8; hosever, only the frequency HL-A8 remained significant in those patients with T cell levels less than 1000 when the value was corrected by the number of specificities tested for. The degree of lymphocytic infiltration of the tumor was found to correlate precisely with the size of the tumor and the level of T cells: Patients with small tumors had a marked degree of lymphocytic infiltrate and high peripheral blood T cell levels; patients with large tumors had absent or minimal lymphocytic infiltration of the tumor with low peripheral blood T cell levels.

Significance to Biomedical Research and Program of the Institute:

The demonstration of depressed cellular immunity in patients with skin cancer suggests that this modality of investigation may be used to serially monitor patients for subsequent recurrence or development of additional tumors if they had presented with a low T cell level initially. Patients who present with high T cell levels should be more vigorously evaluated for the possibility of metastatic disease in regional nodes, and in borderline cases the study suggests that regional lymphadenectomies be done prophylactically. Since cured people with large previous tumors had persisting low T cell levels, this suggests that people with low T cell levels should take adequate precautions against the agents known to induce skin cancer. The studies with histocompatibility typing demonstrate an association between several antigenic frequencies in Celtic patients and the development of skin cancer. This association when taken in conjunction with that found for the depressed cellular immunity may represent the first demonstration in man of the genetic linkage between immune response genes and histocompatibility genes. If subsequent studies confirm this, it would have implications in terms of population screening for subsequent skin cancer development. That the degree of lymphocytic infiltration of the tumor correlates with the peripheral blood T cell levels and the tumor stage demonstrates a correlation between the *in vivo* and *in vitro* studies carried out and would suggest that the degree of lymphocytic infiltration of skin cancer may have prognostic significance for the individual patient.

Proposed Course:

These studies suggest that clinical cancer programs employ *in vitro* and *in vivo* measurements of cell-mediated immunity to assess the patients preoperatively and to follow them postoperatively. Additional patients will be studied with the techniques employed here in an effort to confirm our own results.

Project Description:

Part VII. Alterations of Serum Ribonuclease Levels in Cancer Patients

A. Correlation of Tumor Histology with Ribonuclease Levels

Objectives:

To determine the significance of elevated serum ribonuclease levels in cancer patients. To relate this abnormality with tumor histologic type and stage.

Methods Employed:

Serum alkaline ribonuclease levels were measured by spectrophotometric quantitation of soluble products of RNA digestion by serum ribonucleases. Optimum activity occurred at pH 7.8. AB pooled serum specimens from a single source was used as a constant reference point. Age-correlated criteria of normal ribonuclease levels were determined through a study of serum from over 200 normal subjects. The sera of over 90 cancer patients with a variety of histologic types and stages of malignancies were similarly assayed. All cancer patients were in otherwise good health. Patients with a history of chronic disease or who were taking medications were excluded.

Major Findings:

A significant correlation between serum ribonuclease levels and tumor histologic type was found. Patients with melanomas and sarcomas had ribonuclease levels which as a group did not differ from that of normal controls. Patients with squamous carcinomas and adenocarcinomas, however, had significantly elevated ribonuclease levels. There was no correlation between tumor stage and ribonuclease levels in patients with these latter two types of tumors.

Significance to Biomedical Research and Program of the Institute:

This study demonstrates that patients with squamous carcinomas and adenocarcinomas have significantly elevated alkaline ribonuclease levels. Further study of this phenomenon through assay of the same patients after definitive treatment of their tumors and assay of patients clinically cured of cancer will determine the significance of these findings. If these studies show a return to normal levels after tumor removal of the ribonuclease measured, the use for this assay as a tumor "marker" would appear feasible. Patients with elevated ribonucleases that persist after tumor removal would therefore suggest continued presence of tumor. In such patients, this assay would allow the earlier institution of adjuvant therapy for subclinical residual disease.

Proposed Course:

Continued study of the serum of these patients after definitive tumor treatment and assay of patients clinically cured of tumor are in progress to determine the usefulness of this assay as an indicator of clinically occult tumor.

Project Description:

Part VII. Alterations of Serum Ribonuclease Levels in Cancer Patients

B. The Relation Between Host Immunocompetence and Serum Ribonuclease Levels in Patients with Urologic Cancers

Objectives:

To determine the relationship between ribonuclease activity in the serum of patients with urologic cancer and its relationship to DNCB contact sensitization as an indicator of the competence of the thymus-derived immune mechanism and to relate this abnormality with tumor histologic type and stage.

Methods Employed:

Serum alkaline ribonuclease levels were measured by spectrophotometric quantitation of soluble products of RNA digestion by serum ribonuclease. AB pooled serum specimens from a single source were used as a constant reference point. Each correlated criteria of normal ribonuclease levels. Activity levels were determined through a study of serum from over 200 normal subjects. In this study 42 patients with urologic tumors were studied. Six had bladder tumors, 19 had prostatic carcinoma, 6 had renal cell carcinoma, and 8 had testis tumors, and concurrently 49 adult volunteers were used as age-matched controls.

Major Findings:

This study revealed that serum ribonuclease levels were elevated in 14 of 19 patients with prostatic carcinoma, 6 of 6 patients with renal cell carcinoma, 5 of 6 patients with bladder carcinoma, but only 1 of 8 patients with testis tumors. A significant correlation between SRNase levels and the extent of tumor was demonstrated on patients with prostatic carcinoma but not on patients with other urologic malignant conditions. Moreover, a significant correlation was demonstrated between SRNase and host cell-mediated immunocompetence as measured by delayed cutaneous hypersensitivity to DNCB.

Significance to Biomedical Research and Program of the Institute:

The results suggest that the SRNase assay may be of clinical use in experimental treatment programs to reflect fluctuations in tumor status and host immune status when subtle effects on tumor growth may be obscured by the advanced tumor state.

Proposed Course:

Studies in patients cured of these tumors are in progress to determine whether serum ribonuclease levels return to normal after complete tumor ablation. If so, longitudinal studies will be initiated to determine the relation between tumor treatment and ribonuclease levels in an attempt to establish the clinical usefulness of the assay as a monitor of tumor treatment.

Project Description:

Part VIII. A New Radioassay for the Quantitation of In Vitro Lymphocyte Reactivity

Objectives:

To describe and characterize a new *in vitro* radioassay for the detection of lymphocyte reactivity to phytohemagglutinin (PHA).

Methods Employed:

Normal patients had blood withdrawn and lymphocytes in approximately 100% purity obtained from the collected blood by methyl cellulose sedimentation. Individual leukocyte cultures were made consisting of 5×10^5 lymphocytes in a 1 ml mixture of 80% RPMI 1640 and 20% autologous serum. Phytohemagglutinin-P (PHA) was added at five different concentrations ranging from 0 to 50 μg of PHA/protein per ml of culture. Triplicate cultures with each dose of PHA and controls without PHA were prepared for harvesting at 12 hour intervals up to 84 hours after the addition of PHA. At each time interval either tritiated thymidine or Gallium-67 was added. With thymidine determination the nuclear protein was precipitated with trichloroacetic acid and precipitate processed for scintillation counting. For Gallium-67 each individual culture was washed and then counted in a gamma scintillation counter.

Major Findings:

A correlation between these two methods of determining lymphocyte reactivity was not observed. Thymidine showed maximum reactivity at the time of maximal DNA synthesis; i.e., approximately 60 hours after the addition of PHA. In contrast, the maximum Gallium-67 activity was observed 48 hours after the addition of PHA.

To further determine the site of localization of Gallium-67 within these PHA-reactive lymphocytes, approximately 60 million cells were harvested and prepared for subcellular fractionation studies. The cells were incubated with Gallium-67 in the dose of approximately 1 μc per ml of culture media for 2 hours. The cells were then washed and then homogenized in a Dounce homogenizer. The homogenate was taken up in 5 ml of .30 molar sucrose and layered on top of a discontinuous sucrose gradient of densities 1.04 to 1.27. The gradient was then placed in an ultracentrifuge and centrifuged for 90 minutes at 30,000 r.p.m. The resultant layers were removed and counted in a gamma counter for Gallium-67 activity and also were assayed for protein concentration by the Lowry method. The specific activity of each fraction from the original homogenate was then calculated and specific activity of the different fractions of the homogenate plotted. The results clearly showed association of the isotope within the lysosomal fraction of the homogenate.

Significance to Biomedical Research and Program of the Institute:

These studies would suggest the use of Gallium-67 provides a new radioassay for the detection of PHA-responsive lymphocytes. The materials involved are inexpensive in comparison to the thymidine method and require less man hours and less radiation exposure to the individual. The subcellular fractionation studies would suggest that the binding of Gallium to the lysosome in comparison with previous methods provides a logical and specific binding site for the detection of an organelle which is present in PHA-responsive but not PHA-unresponsive lymphocytes. Thus, a new aspect of immunoreactivity of these lymphocytes may be examined. Therefore, this test can not only give the same information gained from thymidine assays but also may give an additional facet of immunoreactivity not recognized and examined previously.

Proposed Course:

Since this system may provide additional information about the immunologic responsive system within man, it is hoped to extend this system to the study of immunodeficient states as well as immunologic parameters in the oncologic patient. It is felt this will provide a more facile as well as informative system of studying lymphocyte responsiveness to PHA.

Project Description:

Part IX. Isolation and Quantitation of Tumor Associated Antigens (TAA)

A. Lymphocyte Reactivity to Tumor Associated Antigens Isolated from Human Tumors

Objectives:

To examine whether an in vitro test system can detect prior sensitization of lymphocytes by tumor associated antigens; to assess whether cross-reactivity can be detected in the allogeneic system, which would confirm the "common antigen" theory among certain groups of tumors; and to examine the role of humoral factor by varying the serum in the test system. To explore the feasibility of utilizing the test system thus developed to assess the tumor burden, the immunological competence of the host, and to explore the potential of this tool as a guide to cancer therapy.

Methods Employed:

Tumor Associated Antigen Processing: Three methods of antigen processing were utilized. The first was a 3 M KCl extraction method commonly employed. Fresh and stored frozen cancer tissue along with normal tissue, usually muscle from a distant site, was processed to a final concentration of 1 mg/ml and stored in the nitrogen freezer. The preparation was labeled "cancer antigen" and "normal tissue antigen" as control. The second method was a membrane extraction technique, where tumor and normal tissue were first minced, homogenized, frozen, and then exposed to progressively hypotonic saline solution. After high speed centrifugation of the supernatant from previous low speed centrifugation, the pellet was resuspended in normal saline for use following the protein assay. The third antigen extraction technique pertains to the carcinoembryonic antigen (CEA) where the large bowel cancer was processed by the perchloric acid extraction method.

Microtest System: Lymphocytes were isolated by the Ficoll-Hypaque gradient separation method and adjusted to a concentration of 2×10^6/ml in RPMI 1640 culture media. Twenty percent volume of serum of different sources (pooled AB, autologous, and allogeneic) were added to the culture media; 0.1 ml of the lymphocyte suspension and 0.1 ml of four varying concentrations of tumor and normal tissue antigens were added to the wells of the Falcon microculture plates. After 4 to 5 days of incubation the cells were pulsed with tritiated thymidine for 24 hours and harvested, utilizing the automated MASH unit. The lymphocytes were counted for radioactive uptake, which was expressed in disintegrations per minute.

Major Findings:

With four different concentrations of antigens utilized, on many occasions the lowest concentration of 0.001 μg per well gave stimulation to the lymphocytes. Close to 100 patients, mostly preoperative, were studied thus far, and approximately two-thirds of these patients exhibited demonstrable stimulation to tumor antigens at varying concentrations of 0.001 μg, 0.01 μg, 0.1 μg, or 1.0 μg per well, indicating a wide range of active antigenicity of tumors which could not be expressed only in terms of protein concentration. When healthy black lymphocytes were tested against melanoma antigens, a high percentage reacted as strongly as lymphocytes from melanoma patients; whereas healthy white control lymphocytes showed no reactivity to the same antigen. This would be consistent with the Hellstroms' finding in the microcytotoxicity test system. Many of the patients serum when added to the control lymphocytes depressed the reactivity to mitogen, PHA. In the autologous system, lymphocyte "blocking" was also noted.

Cross-reactivity was seen most frequently when CEA was utilized as an antigen. Eleven of 17 colon carcinomas reacted positively as well as a high percentage of breast carcinomas, squamous cell carcinomas, and sarcomas.

Significance to Biomedical Research and Program of the Institute:

Lymphocyte reactivity to tumor associated antigens may be a primary prerequisite for a good prognosis after cancer therapy. Specific immunotherapy in the absence of lymphocyte reactivity may not only be contraindicated but may be harmful if enhancing antibodies are produced. The finding of cross-reacting common antigen is also relevant to immunotherapy. The presence or absence of lymphocyte blocking antibody is of import to assess the immunological state of the patient and in the selection of future therapy. This *in vitro* assay also avoids invasive and hazardous testing such as skin tests with tumor antigens

Proposed Course:

Separation of T and B cells to determine the subpopulation of lymphocytes being stimulated is under way. If certain tumor antigens are found to stimulate predominantly T cells the outcome of immunotherapy will be beneficial. To correlate the course of the disease with the test results is paramount to our future research.

Project Description:

Part IX. Isolation and Quantitation of Tumor Associated Antigens (TAA)

B. Isolation and Characterization of Tumor Associated Antigens from Methylcholanthrene-Induced Tumors in Guinea Pigs

Objectives:

To evaluate the optimal antigen extraction method in which maximal lymphocyte stimulation, delayed hypersensitivity reaction, and immunization to tumor challenge can be accomplished.

Methods Employed:

MCA-25 guinea pig tumor system was studied. Transplanted tumors of early generations were processed utilizing the KCl extraction (Meltzer, Leonard Rapp) and cell membrane extraction (modification of Davies and Mann) methods. These antigens were first used to immunize healthy guinea pigs with complete Freunds adjuvant in two concentrations prepared in 0.1 ml given intradermally. After 3 weeks of immunization given once a week the guinea pigs were challenged with viable single cell suspensions of tumor cells in the 10^7 range. Control animals were given Freunds adjuvant alone in one group and no treatment in another. The second experiment was designed to test the antigen by the delayed hypersensitivity skin reaction in previously sensitized animals. Animals were presensitized with subtake doses of tumor cells and after waiting 2 weeks were tested with intradermal tumor antigen.

Major Findings:

Previously immunized guinea pigs with high dose tumor antigens revealed an enhancing tumor take phenomenon when challenged with lethal dose tumor cells. In the second experiment, animals presensitized with subtake doses of tumor cells, a delayed hypersensitivity type skin reaction was noted in 60% of animals inoculated with high dose range (750 μg) antigen and 20% with low dose range (100 μg) antigen.

Significance to Biomedical Research and Program of the Institute:

The finding of enhancing effect tumor membrane requires caution in application of this modality of therapy in cancer patients. Better methods to secure tumor antigens, to enhance reactivity, to characterize their effect should be sought using this experimental model.

Proposed Course:

Antigen preparations will be modified by column chromatography, by lyophilization, etc., to seek an optimal lymphocyte reactive component. Since guinea pig T lymphocytes form rosettes with rabbit red blood cells, isolation of T lymphocytes is feasible to determine the subpopulation of lymphocytes which react with tumor antigen. These studies are in progress.

Project Description:

Part X. The Denervated Meissner Corpuscle: A Sequential Histologic Study in the Rhesus Monkey

Objectives:

Although the effect of denervation upon nerve trunks has been well established over the last 125 years, the effects of denervation upon the sensory end organs has not previously been investigated. This study will determine by light and electron microscopic investigations the sequential histologic changes in the Meissner corpuscle following sensory denervation of the hand.

Methods Employed:

Three 2 year old Rhesus monkeys had surgical procedures carried out which entailed total sensory denervation accomplished by transecting the median nerve, the ulnar nerve, the dorsal branch of the ulnar nerve, and the superficial sensory branches of the radial nerve at the level of the wrist. This became the operated hand, the non-operated hand became the control hand. Serial biopsies then were taken prior to the surgical procedure and then at subsequent postoperative intervals beginning at 72 hours, then at 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 2 months, 3 months, 4 months, 5 months, 7 months, and 9 months. At each time interval a biopsy was taken from the operated and from the control hand from corresponding fingers. Specimens were submitted in 10% neutral formalin to the histology laboratory where they were stained with Masson and silver staining techniques to demonstrate both connective tissue and neural tissue components of the Meissner corpuscle. In addition, special enzymatic histologic techniques were used. Portions of the specimens were also fixed in Karnofski's fixative and then placed in Cacodilate buffer and submitted for electron microscopic examination. Such specimens were submitted at preoperative time, at 48 hours after the surgery, and at 4 months following the surgery. The finished histologic staining material was submitted as numbered specimens to the Pathology Department, with them being unaware of the source of origin of the submitted material. Following completion of the study, the pathologist's interpretation as to whether the submitted specimens were from an operated or a non-operated hand were then compared with the actual source of the submitted tissue.

Major Findings:

The pathologist identified correctly the source of the submitted specimen in every instance. The criteria by which he was able to determine the operated from the non-operated hand related to the observed sequential histologic changes that occurred in the denervated Meissner corpuscle. These changes were noted as early as the 72 hour specimen in which there was a loss of pink staining material probably reflecting loss of axoplasm within the corpuscle. In the corresponding silver stain the nerve endings by 72 hours were breaking into fragments and degenerating. By 2 weeks following denervation there was complete loss of the lobular architecture of the Meissner corpuscle as seen on connective tissue staining, and this loss of lobular architecture progressed by 4 months to a complete loss of the internal architecture of the Meissner corpuscle. There was a corresponding progressive decrease in overall size of the Meissner corpuscle. This became most marked by 7 months and was considerably diminished in size by 9 months following surgery. The silver stains confirmed at every postoperative interval the absence of nerve terminals from within the Meissner corpuscle. Electron microscopic sections confirmed the light microscopic observations that there was loss of axon terminals from the Meissner corpuscle and progressive diminution in size of the corpuscle with shrinkage of the lamellar cell processes and a relative increase in the interlamellar substance.

Significance to Biomedical Research and Program of the Institute:

The demonstration that denervated sensory end organs undergo progressive histologic changes which eventually lead to considerable alteration in morphology and loss of overall size enables interpretation of previous observations in the nerve-injured patient. For example, it has been observed that when a delay in nerve repair from the time of injury exceeds 4 months the return of sensory function is considerably diminished compared to repairs done at time intervals following injury of less than 4 months. This study would support the concept that long delays following injury permit degenerative changes to occur in the sensory end organs such that when they become reenervated they are incapable of responding to the new axonal terminals with the same capacity they had prior to injury. This suggests that nerve repairs be carried out as quickly after the injury as possible. This study also suggests that in order to replace avulsed skin or tissue loss from the volar surface of the hand this skin should be replaced from a source that contains papillary ridges with the accompanying Meissner corpuscles. Previous experience supports the fact that resurfacing of volar skin with tissue from any other source fails to achieve normal sensation. With specific reference to the National Cancer Institute, this work suggests intervals during which reconstruction of areas involved by tumor should be carried out.

Proposed Course:

The extension of these experiments leads to the same experimental design but with the nerves being immediately sutured following their transection to determine whether reenervating nerves have new Meissner corpuscles develop from their own nerve axons or whether the axons must reenervate the existing Meissner corpuscles at the periphery. Other extension of this work is one in which flaps are used on the finger tips and skin is transferred from a distance to demonstrate the absolute requirement of skin with papillary ridges to supply skin with normal sensation to the finger tip.

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SUMMARY REPORT

RADIATION BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1973 through June 30, 1974

The clinical research program has expanded an already broad scope of studies involving epidemiology, diagnostic methodology, treatment using all major modalities, and rehabilitation. The major new dimension added to ongoing investigations has been the undertaking of studies aimed at defining the immunologic perturbations which coexist with malignant disease, characterizing the immune response (if any) which develops during or following treatment, and exploring the basis for addition of immunologic manipulation as an adjuvant therapy.

The program on Ewing's sarcoma has received national attention, having markedly increased the survival for patients with advanced metastatic disease and given a 5-year survival rate of 50% for the more localized form of this previously dread disease. The presence of an active immunologic response has been documented in some patients and cultures of Ewing's sarcoma cells have now been established *in vitro* as a preliminary step to development of a tumor vaccine for diagnostic studies and potential immunotherapy.

Long-term observation of patients treated for Hodgkin's disease between 1965 and 1969 has continued to yield important data with respect to the diagnostic methods of staging, efficacy of the therapeutic techniques employed, and the implications of the basic disease as well as its treatment for both survival and the quality of life. Our 9-year survival rate for patients with"early" stage Hodgkin's disease is 90%, a figure far superior to that achieved at any other center in the world.

The large-scale study of malignant lymphomas other than Hodgkin's disease has similarly commanded wide attention as witnessed by our extensive invited participation in the first international symposium on this subject during the past year. We have contributed to the understanding of the natural history of these diseases and our pioneering research using small fractionated doses of total body irradiation for treatment has proven a significant therapeutic advance. Based on the 75% survival rate at five years in our pilot clinical trials for advanced lymphosarcoma, total body irradiation using our approach is currently being adopted in many centers throughout the world. Our current investigations with the lymphomas are immunologic in orientation and offer promise of enhancing our basic understanding of the pathogenesis of the lymphomas and of revolutionizing the concepts of therapeutic management.

The development and increased utilization of an Automatic Data Processing system has continued and approximately 500 patients have computerized records at present. A major expansion of the ADP program is envisioned and a comprehensive system is currently being designed to automate the majority of clinical operations in the Branch. This program has been designated as ROMP (Radiation Oncology Management Program) and following completion of the systems design, will hopefully be implemented in a modular fashion during the next several years. The need for a computer assisted operation has become imperative with the annual patient visit rate now exceeding 8000 despite a staff which has shown a minimal numerical increase in the past few years.

The staff of the Radiation Branch has maintained a high level of collaboration with other program areas and has continued to provide considerable advisory support to both intramural and extramural areas. Formal patient consultations numbered in excess of 300 during the past year and thousands of non-clinical radiation exposures of biological materials and animals have been performed, these activities often requiring extensive planning in order to provide high quality support to the clinical and laboratory research of other units.

Serial No. NCI-963
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Immunologic Manipulation on Radiation and Chemical Carcinogenesis and Radiation Response of Tumors

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Walter G. Hardy

Cooperating Units: None

Man Years:

Total:	5/4
Professional:	1/4
Other:	4/4

Project Description:

Objectives: To evaluate the effects of immunologic manipulation on the time of appearance and the incidence of radiation and chemically-induced tumors, and to determine the effect of age at the time of irradiation. Also, to determine the effects of immunologic manipulation on the radiation responsive tumors.

Methods Employed: Total body irradiation or intravenous DMBA in mice with cyclic immunosuppression are the methods utilized to evaluate the alterations and types of tumors and the time of development of radiation and chemically-induced benign and malignant neoplasms. Control groups are expected to reveal differences in spontaneous tumor production under the influence of cyclic immunosuppression. Variations in tumor responsivness to irradiation by non-specific immunologic suppression and enhancement will be correlated with tumor and tumor bed tissue reactions by histopathologic evaluation.

Major Findings: Immunosuppression delays the onset of lymphomas and accelerates the development of carcinomas and sarcomas in both controls and in mice with induction of tumor by irradiation or DMBA. A comparison of age variations in rapidity of tumor development has been striking in preliminary observation. However, the DBA mouse which was used because of availability has proved to be a poor subject for this type of study, and it is now being repeated in Swiss all-purpose mice.

Proposed Course: Complete present carcinogenesis experiments with immunosuppression and in the near future initiate repeat studies with immune stimulation utilizing MSFMB (methanol soluble fraction of mycobacterium butyricum) available from Mason Research Institute. Complete analysis of initial radiation carcinogenesis studies and prepare for publication.

Honors and Awards: None

Serial No. NCI-650 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Service Radiation Therapy

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Kent B. Lamoureux, M.D.; Larry E. Kun, M.D.; Frederick L. Faw, M.S.; Dwight W. Glenn, M.S.; Robert W. Swain, B.S.; Marilyn K. Glover, R.N.; Margaret R. Gable, R.T.; and Janet Rowland, R.N.

Cooperating Units: None

Man Years:

Total:	4/2
Professional:	3/2
Other:	1/2

Project Description:

Objectives: To provide expert radiotherapy consultation and treatment for Clinical Center patients admitted to services other than the Radiation Branch of the National Cancer Institute.

Patient Material: During the twelve month period preceding this report, 225 patients were formally seen in consultation. Advice and management of many additional patients was provided on an emergency or "ad hoc" basis including over 140 logged telephone consultations for advice on treatment or general information.

Methods Employed: The majority of patients were irradiated with the recently installed 6-MeV linear accelerator or cobalt teletherapy following appropriate evaluation and treatment planning as indicated.

Major Findings: Aside from results on research patients as described in project reports from other institutes, the response to radiation therapy has been consistent with that reported from other major centers.

Proposed Course: To continue.

Honors and Awards: None
Publications: None

Serial No. NCI-964 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Cancer of the Upper Air Passages and the Evaluation of Combined Treatment Approaches

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Walter G. Hardy; Ralph E. Johnson, M.D.; Adelbert Evans, Ph.D.; Robert K. Oldham, M.D.; and Diana O'Hagen, R.N.

Cooperating Units: Laboratory of Cell Biology, Division of Cancer Biology and Diagnosis; and the Armed Forces Radiobiological Research Institute.

Man Years:

Total:	5/4
Professional:	2/4
Other:	3/4

Project Description:

Objectives: To evaluate the usefulness of chemotherapeutic agents singly and in combinations as adjuvants to radiation therapy in patients with malignant squamous cell tumors of the mouth and throat.

Patient Material: Patients with post-surgical and post-irradiation recurrences and advanced primary inoperable squamous cell carcinomas of the mouth and throat are accepted for study.

Methods Employed: Drugs currently under study include bleomycin, methotrexate, adriamycin, and vincristine, all of which have shown activity against squamous cell carcinoma. Tumor response to the drugs is evaluated for four to six weeks before initiation of radiation therapy and the drugs are continued during the course of radiation therapy. Great individualization of the radiation is necessary in view of the augmented mucosal reactions from the combined treatment.

Major Findings: An initial study with bleomycin alone and combined with radiation therapy has been completed and sufficient followup has occurred to evaluate early survival data. Currently, a randomized study between bleomycin and methotrexate vs. a four-drug combination combining bleomycin, methotrexate, adriamycin, and vincristine, is under investigation. To date, four patients in each arm have completed therapy, and there is a distinct improvement in early tumor response over bleomycin alone in both instances, but no clear-cut distinction can be seen at the present time between the two arms of the randomization. An example of the efficacy of this adjuvant program is the complete clinical regression of extensive massive tumor of the oral and nasopharynx with only 1800 rads. In the laboratory, bleomycin was found to produce sarcomas at the site of injection in a small group of animals. This was repeated with both subcutaneous and intramuscular injection of bleomycin along with saline-injected controls. The induction of sarcomas has approached ten percent at the primary site. In addition, there is now evidence with long-term followup of immune suppression by bleomycin as evidenced by an earlier appearance of other types of tumors as has been seen with the cyclic immunosuppression by cortisone acetate in the control animals in the carcinogenesis study carried out in Project No. NCI-963.

Proposed Course: Continue the clinical studies with multiple drug adjuvants to radiation therapy. Results in the bleomycin adjuvant study have been presented at a regional cancer meeting, and are being prepared for publication. Analysis of the immune suppression by bleomycin is nearing completion and will be published along with the observations of local induction of sarcomas by local injection of bleomycin.

Honors and Awards: None

Publications: None

Serial No. NCI-684
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973, through June 30, 1974

Project Title: Non-Clinical Service Irradiation

Previous Serial Number: Same

Principal Investigator: Robert W. Swain

Other Investigators: Gilbert M. Bowie, Laboratory of Physiology, NCI

Cooperating Units: Laboratory of Physiology, NCI

Man Years:

Total:	3/10
Professional:	1/10
Other:	1/5

Project Description:

Objectives: To provide facilities for the performance of irradiation of biological materials and animals as required for research studies by investigators outside of the Radiation Branch. This includes consultation as to methods and types of irradiation and the preparation of material.

Methods Employed: A dual unit 250 Kvcp x-ray generator is provided and maintained in proper operating condition. During the past year, an instruction book was prepared to enable investigators and technicians to operate the unit properly by themselves.

Proposed Course: Due to the age of the equipment (28 years) and difficulty in obtaining replacement parts, it is recommended that these units be replaced with more modern equipment.

The services will continue to be offered.

Honors and Awards: None

Publications: None except as indicated by the use of these facilities in publications from other laboratories.

Services Provided Using the 250 KVCP Double Header Westinghouse Quadrocondex from April 1, 1973 to April 1, 1974

Serial No. NCI-684

Organization	Number of Exposures
A-A&R	67
A-LI	504
A-LPB	10
C-BGY	69
C-CG	3
C-CH	17
C-DE	49
C-DR&D	20
C-EXP	67
C-GL&C	64
C-HTCB	107
C-I	607
C-CB	13
C-LBGY	107
C-LCI	29
C-LMP	102
C-LP	2
C-LPHY	981
C-LTCB	6
C-M	9
C-MET	102
C-PO	4
C-R	100
C-Surg	129
C-VB	5
C-VLL	33
CH-BB	66
D-HG	399
D-LM	211
I-LCI	13
I-LI	230
I-LM	7
I-LMI	228
I-LPD	3
I-LVD	28
I-LVR	29
N-LNNS	4
Miscellaneous	111

*BIONETICS	126
*MELOY	156
*MICROBIOLOGIC	446
*FLOW	5
	5268

Exposures for Microbiologic, Bionetics, Meloy and Flow laboratories were performed on materials for projects under contract to the National Cancer Institute.

Materials irradiated included cells, tissue cultures, blood, mice, rats, hamsters, chickens, and guinea pigs. Exposures ranged from 100 R to 15000 R.

Serial No. NCI-967
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Measurement of "W" for 14 Mev Neutrons

Previous Serial Number: Same

Principal Investigator: Dwight W. Glenn, M.S.

Other Investigators: A. P. Wheatley

Cooperating Units: None

Man Years:

Total:	1
Professional:	3/4

Project Description:

Objectives: To obtain a value of "W" for neutron beams to enable the Bragg-Gray theory to apply to ionization measurements, i.e., relate ionization in a material to the absorbed dose in that material.

Methods: The absorbed dose will be measured in a tissue-equivalent material by determining directly, the amount of energy deposited. The amount of energy deposited will be related to the amount of ionization measured in a similar material. (Calorimeter compared to ionization in a tissue-equivalent gas-filled ionization chamber.)

Major Findings: Test exposures with cobalt irradiation indicate stability of system and feasibility to proceed with neutron irradiation.

Significance for Cancer Research: Obtaining the value for "W" for neutrons will assist in more accurately determining and comparing tumor doses to patients undergoing radiation treatment with neutron beams. This work would fit Objective 6, Approach 1, of the National Cancer Plan in developing methodologies of radiation.

Proposed Course: Further cobalt irradiation will be utilized to study the "heat defect" phenomena with tissue equivalent plastic prior to moving the system to the Naval Research Laboratory Cyclotron facility. At the cyclotron facility, the neutron beam presently used for patient treatments will be measured. This will be followed by comparing doses from the

Texas A. & M. cyclotron neutron beam. These neutron exposures should result in sufficient data to calculate "W" for two various neutron beams while serving as a dose comparison between two major facilities that are treating patients with neutron beams.

Honors and Awards: None

Publications: None

Serial No. NCI-975(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of Tumor Biology and Diagnostic Evaluation in Lung Cancer

Previous Serial Number: Same

Principal Investigator: Kent B. Lamoureux, M.D.

Other Investigators: Ralph E. Johnson, M.D.

Cooperating Units: Laboratory of Pathology, NCI
Diagnostic Radiology and Nuclear Medicine, NHLI, CC, NIH

Man Years:

Total: 3/2
Professional: 2/2

Project Description:

Objectives: The purpose of this study is to improve understanding of the natural history of lung cancer by means of extensive diagnostic evaluation at the time of diagnosis and serially throughout the course of disease. Another purpose of the study is to ascertain the proper timing of radiotherapy within a cyclical chemotherapeutic program.

Patient Material: Approximately 50 patients have been entered into this program.

Methods Employed: Exhaustive diagnostic evaluation of each patient has been undertaken using both standard and investigational techniques and these studies have been repeated and correlated with clinical findings and developments for estimation of reliability. Combination chemotherapy utilizing cytoxan, vincristine, and adriamycin has been used prior to radiotherapy and response rates to this combination are being judged. The effectiveness of the subsequent radiotherapy is also being evaluated.

Major Findings: Results are currently being tabulated and itemized for computer programming. The following statements appear to be probably correct.

1. Symptomatic radiation reactions in the lung appear to be increased with related chemotherapy.

2. Copper, ceruloplasmin, and fibrinogen levels may be of prognostic value when looking for metastases.

3. Alkaline phosphatase levels have no correlation with presence or absence of bone metastases.

4. Prophylactic brain irradiation appears to be very worthwhile in preventing serious CNS impairment.

5. Combination chemotherapy may induce a resistance to subsequent radiotherapy.

Proposed Course: Present results are to be summarized and evaluated resulting in accession of more patients into a modified study to further elucidate the above major findings.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A JOURNAL

Lamoureux, K. B.: Diagnostic problems after radiotherapy involving the lung. Southern Med. J. 66: 1388-1392, 1973.

Serial No. NCI-987(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Automatic Data Processing of Clinical Research

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Thomas C. Pomeroy, M.D., Frederick Faw, M.S.,
Janet Rowland, R.N., Michael Stump, B.S.

Cooperating Units: Biometry Branch, NCI

Man Years:

Total:	3/2
Professional:	2/2

Project Description:

Objectives: Development and implementation of an ADP system for management of clinical research data.

Patient Material: Patients entered into protocol studies are having their clinical records reviewed, coded, and stored on tape at the present time. Approximately 500 patients with Hodgkin's disease, malignant lymphoma, and Ewing's sarcoma have computerized records which are kept updated by continuous editing using an on-line terminal.

Methods Employed: Programs have already been developed by the staff of the Radiation Branch utilizing the Wilbur program and the DCRT IBM-360 computer. A system design is currently underway to expand computer assistance to all major facets of our clinical operation.

Major Findings: This program has greatly facilitated the accuracy of our protocol studies by insuring the acquisition of clinical data and maintaining the data in a readily retrievable mode. Most large scale analyses of results are now dependent upon this ADP system.

Proposed Course: Continued expansion of the program to all ongoing clinical studies and investigation of methods for facilitating the extrapolation of such a system to general use at NIH and in the medical community.

Honors and Awards: None

Publications: None directly.

Serial No. NCI-988(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Chronic Lymphocytic Leukemia

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Ursula Rühl, M.D., Costen Berard, M.D., and Harvey Gralnick, M.D.

Cooperating Units: Laboratory of Pathology, NCI
Hematology Department, Clinical Pathology Department, CC

Man Years:

Total:	1/1
Professional:	1/1

Project Description:

Objectives: This study is directed toward improving the understanding of the epidemiology and natural history of chronic lymphocytic leukemia.

Patient Material: Approximately 100 patients with chronic lymphocytic leukemia have been seen in the Radiation Branch and are available for study.

Methods Employed: Information is currently being collated and coded for computer storage, retrieval, and analysis.

Major Findings: Description of major findings awaits analysis of data once computerization of information has been completed.

Proposed Course: Continued long-term observation of patients entered on prospective treatment protocols is anticipated but no significant number of additional patients will be accessioned into study.

Honors and Awards: None

Publications: Oldham, R. K., Larson, S. M., Givelber, H. M., and Johnson, R. E.: A preliminary study of ^{51}Cr-Labeled platelets for evaluation of splenic sequestration in chronic lymphocytic leukemia. J. Nucl. Med. 14: 219-222, 1973.

Whang-Peng, J., Gralnick, H. R., Johnson, R. E., Lee, L. C., and Lear A.: Chronic granulocytic leukemia (CGL) during the course of chronic lymphocytic leukemia (CLL): Correlation of blood, marrow, and spleen morphology and cytogenetics. Blood 43: 333-339, 1974.

Serial No. NCI-989(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Hodgkin's Disease

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Clinical Associates

Cooperating Units: Medicine Branch, Surgery Branch, and Laboratory of Pathology, NCI
Diagnostic Radiology and Nuclear Medicine, CC, NIH

Man Years:

Total: 2/2
Professional: 2/2

Project Description:

Objectives: To understand the natural history of Hodgkin's disease, assess the reliability of diagnostic methods used to guide treatment, examine the effectiveness of radiotherapy and chemotherapy used singly and in combination, and to document the long-term results of treatment and the effects of both treatment and the underlying disease process on the quality of survival.

Patient Material: Two hundred patients with previously untreated Hodgkin's disease were entered into prospective protocol studies beginning in 1965. Accession of patients to the study has now been terminated.

Methods Employed: A collaborative effort involving many cooperating units was directed toward each patient to systematically evaluate the biologic characteristics of Hodgkin's disease, the methods used for diagnostic study, the accuracy of various staging procedures, and comparative clinical trials were undertaken to test the efficacy of various therapeutic techniques.

Major Findings: These have been detailed in the publications listed below.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A BOOK

Johnson, R. E.: Total nodal irradiation of Hodgkin's disease: Technical and clinical aspects. In Fletcher, G. H. (Ed.): Textbook of Radiotherapy, Philadelphia, Lea and Febiger, 1973, pp. 525-544.

Johnson, R. E.: Radiation management of lymphomas. In Seventh National Cancer Conference Proceedings, Los Angeles, California. Philadelphia, J. B. Lippincott Co, 1973, pp. 375-378.

Johnson, R. E., and Kun, L. E.: Influence of histology on treatment selection and prognosis for Hodgkin's disease and malignant lymphomas. In Frontiers of Radiation Therapy and Oncology. Basel, Switzerland, S. Karger Publishers, Vol. 8, 1974.

Johnson, R. E.: Radiotherapy of Hodgkin's disease and the malignant lymphomas. In Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, Springer-Verlag, Vol. 46, 1974.

ARTICLES PUBLISHED IN A JOURNAL

Milder, M. S., Larson, S. M., Bagley, C. M., DeVita, V. T., Johnson, R. E., and Johnston, G. E.: Liver-spleen scan in Hodgkin's disease. Cancer 31: 826-834, 1973.

Lamoureux, K. B., Jaffe, E. S., Berard, C. W., and Johnson, R. E.: Lack of identifiable vascular invasion in patients with extranodal dissemination of Hodgkin's disease. Cancer 31: 824-825, 1973.

Young, R. C., DeVita, V. T., and Johnson, R. E.: Hodgkin's disease in childhood. Blood 42: 163-174, 1973.

Johnson, R. E., Kun, L. E., Belladonna, J. A., Johnson, S. K., Brereton, H. D., and Cohen, G. A.: Hematologic recovery and deterioration after "successful" radiotherapy for Hodgkin's disease. Ann. Intern. Med. 80: 213-216, 1974.

Johnson, R. E., Thomas, L. B., Johnson, S. K., and Johnston, G. S.: Correlation between abnormal baseline liver tests and long-term clinical course in Hodgkin's disease. Cancer. (In Press)

Canellos, G. P., DeVita, V. T., Arseneau, J. C., and Johnson, R. E.: Carcinogenesis by cancer chemotherapeutic agents: Second malignancies complicating Hodgkin's disease in remission. Ann. N.Y. Acad. Sci. (In Press)

Brereton, H. D., and Johnson, R. E.: Calcification in mediastinal lymph nodes after radiotherapy of Hodgkin's disease. Radiology (In Press)

Serial No. NCI-990(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Non-Hodgkin's Lymphomas

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Vincent T. DeVita, M.D., Paul B. Chretien, M.D., Costen Berard, M.D.

Cooperating Units: Medicine Branch, Surgery Branch, and Laboratory of Pathology, NCI

Man Years:

Total: 10/2
Professional: 6/2

Project Description:

Objectives: To increase our understanding of the natural history of the malignant lymphomas, to assess the methods of diagnostic evaluation including histopathology, and to evaluate therapeutic approaches via carefully designed and prospective clinical trial.

Patient Material: Approximately 250 patients have been accessioned into this collaborative study.

Methods Employed: Extensive diagnostic and immunologic profiles are compiled prior to initiation of therapy and continued serially during the treatment and post-treatment phase. Various new treatment technicques are being researched in controlled clinical trials.

Major Findings: The most significant results have pertained to defining the natural history of these diseases, and to the establishment of total body irradiation using small fractionated doses as a valuable modality of treatment for selected histologic subtypes of lymphoma, irrespective of the clinical extent of involvement.

Proposed Course: The studies are being continued and expanded with respect to the immunologic aspects of the program.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A BOOK

Johnson, R. E., and Kun, L. E.: A discussion of the influence of histology on treatment selection and prognosis for Hodgkin's disease and malignant lymphomas. In Frontiers of Radiation Therapy and Oncology. Basel, Switzerland, S. Karger Publishers, Vol. 8, 1974.

Johnson, R. E., Chretien, P., O'Conor, G. T.: Influence of extensive biopsy samply at laparotomy on planning of radiotherapy in non-Hodgkin's lymphomas. In Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, Springer-Verlag, Vol. 46, 1974.

Johnson, R. E.: Radiation management of lymphomas. In Seventh National Cancer Conference Proceedings, Los Angeles, California. Philadelphia, J. B. Lippincott Co., 1973, pp. 375-378.

Johnson, R. E.: Radiotherapy of Hodgkin's disease and the malignant lymphomas. In Recent Results in Cancer Research: Diagnosis and Therapy of Malignant Lymphomas. Berlin, Germany, Springer-Verlag, Vol. 46, 1974.

ARTICLES PUBLISHED IN A JOURNAL

Johnson, R. E.: Radiation therapy of generalized lymphocytic lymphomas. Amer J. Roentgenol. Radium Ther. Nucl. Med. 117: 50-53, 1973.

Bagley, C. M., Thomas, L. B., Johnson, R. E., Chretien, P. B., and DeVita, V. T.: Diagnosis of liver involvement by lymphoma: Results in 96 consecutive peritoneoscopies. Cancer 31: 840-847, 1973.

Johnson, R. E., Chretien, P. B., O'Conor, G. T., DeVita, V. T., and Thomas, L. B.: Radiotherapeutic implications of prospective staging in one hundred consecutive cases of non-Hodgkin's lymphoma. Radiology 110: 655-657, 1974.

Johnson, R. E.: Total body irradiation (TBI) as primary therapy for advanced lymphosarcoma. Cancer. (In Press)

Johnson, R. E.: The management of generalized malignant lymphoma with "systemic" radiotherapy. Br. J. Cancer. (In Press)

Canellos, G. P., DeVita, V. T., Young, R. C., Chabner, B. A., Schein, P. S., and Johnson, R. E.: Therapy of advanced lymphocytic lymphoma: A preliminary report of a randomized trial between combination chemotherapy (CVP) and intensive radiotherapy. Br. J. Cancer. (In Press)

Chabner, B. A., Johnson, R. E., Chretien, P., Schein, P.S., Young, R. C., Canellos, G. P., Hubbard, S. H., and DeVita, V. T.: Percutaneous liver biopsy, peritoneoscopy, and laparotomy: An assessment of relative merits in the lymphomas. Br. J. Cancer. (In Press)

Johnson, R. E., DeVita, V. T., Kun, L. E., Chabner, B. A., Chretien, P. B., Berard, C. W., and Johnson, S. K.: Patterns of involvement with malignant lymphoma and implications for treatment decision making. Brit. J. Cancer. (In Press)

Serial No. NCI-6502 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Study of Ewing's Sarcoma as a Model System in Experimental Therapeutics for Adjuvant Chemotherapy

Previous Serial Number: Same

Principal Investigators: Thomas C. Pomeroy, M.D.

Other Investigators: Ralph E. Johnson, M.D., Robert K. Oldham, M.D., Adelbert Evans, Ph.D., and Diana O'Hagen, R.N.

Cooperating Units: Laboratory of Cell Biology, Division of Cancer Biology and Diagnosis; and the Armed Forces Radiobiological Research Institute.

Man Years:

Total:	5/4
Professional:	4/4
Other:	1/4

Project Description:

Objectives: To determine the value of adjunctive chemotherapy in the management of Ewing's sarcoma, both presenting initially with no evidence of clinical metastasis and in patients with disseminated disease at the time of admission. The evaluation of glycoprotein profiles as an indicator of total tumor burden will be studied in conjunction with the laboratory at the Armed Forces Radiobiological Research Institute. Evaluation of immune competence and the potential for non-specific and specific immunotherapy will be carried out with studies available through the Laboratory of Cell Biology.

Patient Material: Patients with and without known metastatic disease will be admitted to this study.

Methods Employed: Adjuvant therapy with active chemotherapeutic agents is combined with primary radiation therapy and prophylactic irradiation of the central nervous system. Evaluation of immune competence is being studied by the use of microcytotoxicity assays of the patients' lymphocytes against Ewing's cell lines and other cell lines. T-rosetting, lymphocyte stimulation and macrophage inhibition factors are also included. In the future, serum that is now being stored will be evaluated for the presence of blocking antibodies. The determination of glycoprotein profiles will be obtained serially on all patients initially, during treatment, and followup after treatment.

Major Findings: Five-year survival rates in patients presenting without any evidence of clinical metastasis have exceeded fifty percent as contrasted with the expected survival of approximately ten percent ten years ago. There has been a similar improvement in disease control in patients presenting with metastasis since the addition of adriamycin into the protocol which was initiated in November, 1971. Current experience with the adriamycin protocol in the prophylactic treatment indicates a potential for greater than seventy percent five-year survival. Preliminary evidence indicates that the immune competence determinations may provide prognostic information as well as provide some method of selection for addition of non-specific and/or specific immunotherapy. Glycoprotein profiles have shown some correlation with continued presence of active tumor and in some instances have predicted relapse a few months before the relapse was clinically evident.

Proposed Course: Continue patient acquisition for further evaluation of treatment methods and to provide serial laboratory evaluation of immune competence and further evaluation of glycoprotein profiles as a measure of total tumor load. The initiation of non-specific and specific immunotherapy is planned for patients with high risk of early relapse.

Honors and Awards: None

Publications: Frankel, R.S., Jones, A.E., Cohen, J.A., Johnson, K.W., Johnston, G.S., and Pomeroy, T.C.: Clinical correlations of ^{67}Ga and skeletal whole-body radionuclide studies with radiography in Ewing's sarcoma. Radiology 110:597-603, 1974.

Johnson, R.E., and Pomeroy, T.C.: Evaluation of therapeutic results in Ewing's sarcoma. Am. J. Roentgenol. Radium Ther. Nucl. Med. (Submitted for publication).

Pomeroy, T.C., and Johnson, R.E.: Prognostic factors for survival in Ewing's sarcoma. Am. J. Roentgenol. Radium Ther. Nucl. Med. (Submitted for publication).

Pomeroy, T.C., and Johnson, R.E.: Combined modality therapy of Ewing's sarcoma. Cancer (Submitted for publication).

SUMMARY STATEMENT
ANNUAL REPORT
DERMATOLOGY BRANCH
DCBD, NCI
July 1, 1973 through June 30, 1974

The Dermatology Branch conducts both clinical and basic research on the etiology, diagnosis, and treatment of malignant diseases involving the skin and the host's response to these diseases. The Branch also serves as dermatologic consultant to all other services at the Clinical Center (approximately 800 patients are seen in consultation per year) and to NIH employees through the Employee Health Clinic. The Branch currently includes the Branch Chief, 4 senior investigators, 2 staff fellows, 1 visiting fellow, 5 clinical associates and 3 senior investigators (Not To Exceed 1 year).

The Dermatology Branch has made significant contributions toward the objectives of the National Cancer Plan. The major research achievements of the Dermatology Branch for the past year are as follows:

Xeroderma pigmentosum. This rare autosomal recessive dermatologic disease is characterized by pigmentary changes, telangectasia, atrophy, and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients (basal and squamous cell carcinomas, malignant malanomas, etc.) are histologically identical with those found in otherwise normal persons. A defect (presumably in an endonuclease) in repair of UV damage to DNA has been found in XP cells. As suggested by the formulators of the National Cancer Plan (Executive Report, Vol. II, Section 1-3. p. II 14), "development of this important area may lead to an understanding of the basic nature of the carcinogenic change...and to the development of effective preventive measures." The NCI Dermatology Branch has the largest series of XP patients on whom complete photobiological, clinical, and laboratory evaluations have been performed. Cells from several tissues have been obtained from these patients, and work in this Branch has demonstrated the suitability of cells other than the customarily used skin fibroblasts for studying the DNA repair defect in XP. Thus, use of peripheral blood lymphocytes rather than dividing cells largely overcomes the problem of the repair synthesis being obscured by semiconservative DNA synthesis. Investigators in the Dermatology Branch have discovered a form of XP in which the patient's cells have no demonstrable DNA repair defect, despite the presence of severe skin disease including numerous malignant tumors. Cells from this patient and other XP patients are being studied in the Branch to determine the role of DNA repair in tumor formation after exposure to ultraviolet radiation of chemical carcinogens which react with and distort DNA.

Genetic complementation studies are being performed by fusing cells from pairs of XP patients. The work of last year, in which 4 complementation groups had been discovered, has now been extended to other lines of XP cells in collaboration with the laboratory of Prof. Bootsma of the Netherlands. Prof. Bootsma's group had reported finding three complementation groups among their XP cells lines. Studies in each of our laboratories determined that there are at least five XP complementation groups suggesting that defects arising in any one of at least five genetic loci can give rise to XP.

In addition studies are being conducted in cooperation with Dr. Rufus Day, NCI investigating the relationship between the rate of UV-induced DNA repair as measured by thymidine incorporation and the ability of XP cells to repair UV-irradiated adenovirus. These studies are important since they compare quantitative differences in repair with evaluation of the accuracy of repair. With this system, patients in various complementation groups are being studied, in an effort to obtain further evidence for the role of XP abnormal DNA repair in human carcinogenesis. In addition an effort is being made to determine the difference between fast and slow components in the repair process which may be of some importance in evaluating XP.

There has been an attempt made to establish long-term lymphoblastoid lines from XP patients' peripheral blood. The existence of such long term cultures would make possible many experiments now not easily performed with cultured fibroblasts. The relationship between ageing and ability to repair DNA damage is being explored in a study of cell passage number and effects on UV-repair abilities of cells aged in vitro. Studies are being performed on the effect of UV-irradiation on chromosome number and structure in XP cells. Preliminary results indicate chromosomal abberations to be more frequent in XP cells. Finally, ligase activity is being measured in XP cells from various complementation groups.

Cells from all XP patients studied by the Branch are characterized according to their DNA repair rate, and the patients undergo a thorough clinical evaluation. The cells, together with the clinical classifications, are sent to the American Type Culture Collection for dissemination for use by other investigators.

Malignant Melanoma. Malignant melanoma is a highly malignant tumor in which production of a specific differentiated cell product, melanin, not only continues but is often increased. The melanin granules in tumors are, however, morphologically distinguishable from those found in normal cells. The Dermatology Branch is investigating melanogenesis and its regulation in normal and malignant cells to determine the relationship of this process to melanoma formation; cells from various pigmentary mutants of the mouse have been compared to elucidate the genetic basis for control of malanin production. It has been found that (1) Melanogenesis in normal black mice and the more than 10 pigment mutants studied, followed a common pathway of melanosome formation; the major difference noted among the mutants was the premature termination of melanization of the granules at different stages of melanosome formation; (2) Melanosomes from black mice contain seven proteins, six of which are of extremely high molecular weight. Preliminary results of analysis of malanosomes from amphibians are remarkably similar to those of the mouse, although proteins from (mouse) melanoma melanosomes are quite different from normal - a most interesting observation. We are currently preparing to isolate and characterize melanosomes from normal and malignant human tissue; and (3) Tyrosinase activity in black mice seems to be under the control of a heat-labile, low molecular weight (dialyzable) inhibitor. Tyrosinase activity in albino mice, heretofore assumed to be absent, can be demonstrated when tissue homogenates are pretreated with detergents. This indicates that the c locus (albino) should be regarded (in mice) as a regulator locus rather than a structural locus. In addition, we have determined that melanogenesis in the mouse in dependent upon tyrosinase activity rather than peroxidase activity, as has been suggested by other workers. These studies are currently being expanded to include normal and malignant pigmentary systems in humans, as well as in mice. (4) It has been found that tyrosinase activity is under allosteric control and that phospholipids play an important role in the expression of the enzyme's activity. It has also been found that allosteric regulation in murine melanomas is altered, perhaps explaining the atypical melanosome formation in these tissues. (5) Finally, it has been found that some proteins of malignant melanoma melanosomes are unique and not found in normal melanin granules. These abberant proteins found in malignant tissue will hopefully be taken advantage of in developing an immunotherapeutic approach against melanomas.

T-Cell Leukemia (Sezary Syndrome) Another major area of research in the Dermatology Branch is the study of the Sezary syndrome, a lymphocytic leukemia of the T cell type. The Sezary syndrome is a lymphomatous disease characterized by intense generalized reddening and exfoliation of the skin, along with leukemic cells circulating in the blood. Ultrastructural studies in the branch, have shown the large circulating cells to have a characteristic convoluted nucleus. In addition the Sezary cell has been shown to be lymphocytic by virtue of PHA responsiveness in culture and has been shown to have chromosomes abnormal in both number and structure. Using quantitative measurements of nuclear contour index (NCI) and cell diameter it is possible to quantitate the nuclear irregularity of the Sezary cell in comparison to other abnormal leukemic cells. It has been found that a cell with a nuclear contour index above 8.6 in the circulating blood is a Sezary Cell. (Malignant T-lymphocyte). Projects are underway to determine whether or not any individual lymphocyte can be identified as a B or T lymphocyte on the basis of its nuclear contour index. Additional new information concerning the pathogenesis and treatment of the Sezary syndrome have been obtained and new projects are underway in attempts to further delineate this disease:
(1) T cells, of at least certain morphologic varieties, preferentially infiltrate the skin and spare the bone marrow;
(2) If the Sezary syndrome is redefined as T cell leukemia with associated erythroderma, we have now identified three morphologic variants;
(3) Each patient in this expanded Sezary group differs from the other patients at the cellular level, using functional, antigenic and morphologic criteria. In addition, several patients had unique clinical features, i.e., one patient's inverse cyclicity and an other's recurrent hepatic and renal infiltration;
(4) The first case of lymphocytic leukemia bearing both B and T cell markers on the same cells was identified;
(5) Review of the cumulative NIH experience with the Sezary syndrome revealed a remarkable lack of thymic infiltration at autopsy. This in addition to the identification of T cells in the skin of our more recent patients suggests that the skin may actually be playing a role in the aberrant differentiation of these abnormal cells;
(6) An affinity of T cells for the skin is supported by our observation that the large majority of cells in mycosis fungoides, lichen planus, Mucha Habermann's disease, parapsoriasis en plaque and lymphomatoid papulosis also appear to be T cells;
(7) One patient with T cell leukemia and tumor stage mycosis fungoides superimposed on a generalized erythroderma was treated with leukapheresis, a

process by which leukocytes are selectively removed from the buffy coat in a continuous flow centrifuge, and the cutaneous lesions resolved. The patient returned 100 days later for a repeat of the leukapheresis with similar results;

(8) Cells from one patient with the Sezary syndrome have been maintained in culture for one month and retain both their receptors for sheep erythrocytes and their distinctive morphology;

(9) An immunotherapy protocol has been devised and accepted for use. However, because of the relative success of our more conservative therapeutic approaches, immunotherapy has not yet been required. Other approaches include the present trial run of L-asparaginase which appears to be of at least limited efficacy;

(10) Investigation at the cellular level in the patients with the Sezary syndrome includes efforts to reconstitute abnormal responses to mitogens and in mixed leukocyte cultures with soluble factors, utilization of these cells in cell mediated lympholysis, isolation of the product of the mixed leukocyte reaction locus, and determination of the effects of various lymphokines on the *in vivo* and *in vitro* parameters of the disease.

Study of Cell Mediated Immunity and Contact Sensitization in Human Skin. The sensitization of human skin with the chemical dinitrochlorobenzene (DNCB) has become a very frequently used means of testing the ability of humans to generate a cell mediated immunity response. In our laboratory an *in vitro* lymphocyte transformation technique has been developed which parallels the development of contact sensitization of human skin. Using these techniques we have been able to demonstrate a temporal relationship between *in vivo* reactivity and *in vitro* specific lymphocyte transformation during primary immune response. A peak in *in vitro* activity is observed two to four weeks following the application of a primary sensitizing dose of DNCB, followed by a fall off to lower levels within two months following primary sensitization. These sequential studies of allergic contact dermatitis in humans are the first such studies of the kinetics of the primary cellular immune response using both *in vivo* and *in vitro* techniques. Additional studies have demonstrated the quantitative relationship between the intensity and duration of allergic contact dermatitis to DNCB *in vivo* and the magnitude of specific lymphocyte transformation *in vitro*. It has also been found that repeated challenges of DNCB boosts very specifically *in vitro* lymphocyte transformation. Also, a lymphokine activity in allergic contact dermatitis has been discovered by using the *in vitro* techniques described above, and there is evidence that such lymphokines may be mediators of cell mediated immunity. The time course of release of this lymphokine in human peripheral blood cultures has been demonstrated and reproduceable dose response curves for this lymphokine activity have been demonstrated. This offers a biological system with great potential for the characterization and isolation of the mediators of cell mediated immunity. Preliminary data on the characterization of the DNCB induced lymphokines indicate that they are lyophilizable, heat labile, stable in polyacrylamide gels and further studies are under way to chemically characterize this substance. These studies are especially important since previous investigations of cell mediated immunity using microbial antigens lacked effective control in contrast to exquisite controls maintained in the DNCB sensitization system. The *in vitro* assay, since it is specific and quantitative makes it an invaluable tool for measuring lymphocyte activation under a variety of experimental conditions.

Chemistry and Biosynthesis of Mammalian Epidermal Tonofilaments. Preliminary procedures have been worked out for the isolation of tonofilaments from bovine epidermis. The polypeptide chains that comprise tonofilaments may be isolated directly from the epidermal tissues. This is achieved by extraction of tissue in 8M urea buffer containing 2-mercaptoethanol. A procedure for separation of these polypeptide chain has been established using chromatographic methods. Tonofilaments are a major component of epidermal cells and little or nothing is known about their chemistry or function; therefore, it is important to learn more about these structures in order to better understand normal epidermal cells as a baseline for studying malignant degeneration into basal cell and squamous cell carcinomas which probably are the two most common cancers occurring in man.

Vitamin A and Epidermal Differentiation. Using S^{35}sulfate and tritiated fucose, it has been found that Vitamin A stimulates the incorporation of sulfate and fucose into epithelial mucin of embryonic chick skin. Contrary to what would be expected from others' findings it has been determined that Vitamin A rather than stimulate DNA synthesis, in our system, inhibits the synthesis of DNA in chick skin. Using the scanning microscope it has been found that the scale formation in the leg of the chick embryo is inhibited, as has been previously shown for feather formation in the chick by others.

Scanning and Transmission Electron Microscopy. The structural parameters of cell function and disease are being studied with a wide range of optical methods comprising light microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). These observations provide: (1) the most detailed descriptive morphological data available with current technology; (2) direct experimental evidence for the physiological roles of certain cells and organelles, and (3) correlative evidence and controls on purity and sampling of cells or cell components being studied by other methods. The development, behavior, fate, and pathology of leukocytes and epithelial cells are our principal concern; these cells play an essential role in normal and diseased skin, and are uniquely suited for experimental study.

Using methods which enable scanning microscopy to be performed on cells previously stained and identified has been proven useful in studies of a wide variety of cells in tissue culture-cells such as; chicken embryo cells, fibroblast cell lines, melanoma cells, renal carcinoma cells and cells from pleural effusions of breast cancer patients. Distinctive surface morphology has been found to occur only in a few cell lines while considerable variations characterize most of the cultured cells examined.

A new procedure employing both scanning electron microscopy and light microscopy has been developed in order to visualize non-attached cells fixed in suspension and recovered in high yield. The importance of visualizing cells fixed directly in suspension is emphasized by the striking morphologic changes observed in these cells when they are allowed to settle for only 1 minute - cells become flattened and assymetrical and ruffles disappear from the visible surface of the cells.

T and B lymphocytes have been examined under the scanning electron microscope to attempt confirmation of observations by other investigators that T cells tend to have smooth surfaces and B cells ruffled surfaces. We now suspect from preliminary studies that the smooth surface forms described by others as distinctive of T lymphocytes are artifactual and T and B lymphocyte populations from nylon columns appear to be indistinguishable by the scanning electron microscope.

Also it has been claimed that cells in metaphase could be recognized by their round shape and the smoothness of their surface; however, using a method which first stains the cell nuclei with feulgen to identify the cell cycle phase it has been determined by scanning microscopy that the surface features and shape of these cells are not consistant with any phase of the cell cycle including metaphase.

Serial No. NCI-3613
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Extraction, Isolation and Characterization of Keratohyalin Granules

Previous Serial Number: Same

Principal Investigator: Walter G. Klingler, M.D.

Man Years: (July 1, 1973 through June 30, 1974)

Total:	1.1
Professional:	0.3
Other:	0.8

Project Description:

Objectives:

1) To isolate and characterize human and rat keratohyalin.

2) To demonstrate the histochemical, ultrastructural and biochemical properties of human and rat keratohyalin.

3) To elucidate similarities and differences of keratohyalin from various animal species by isolation and subsequent biochemical and immunological characterization.

4) To develop a short-term organ culture system for bovine epidermis.

5) To isolate bovine keratohyalin synthesized in organ culture utilizing tritiated amino acid precursors as radiochemical markers.

6) To monitor the effect of various pharmacologic agents on the synthesis of bovine keratohyalin, a specific epidermal marker protein in short-term organ culture.

7) To study the physiology of bovine keratohyalin synthesis in organ culture by employing metabolic blocking agents (e.g. puromycin) and agents which may stimulate protein production (e.g. dibuitryl cyclic AMP).

8) To apply the short-term organ culture of bovine epidermis to address basic questions regarding function of keratohyalin.

The Objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

1) Human and rat tissue are fixed routinely and studied histochemically and ultrastructurally. Extraction patterns are determined as previously described using serial dilutions of various salt solutions.

2) Human and rat epidermis are prepared and extracted in 1.0 M potassium phosphate buffer. Aggregates are formed by dialysis against distilled water; these aggregates may be fixed routinely and studies histochemically and ultrastructurally or solubilized in sodium decyl sulfate and subjected to disc gel electrophoresis.

3) Bovine epidermis is prepared as previously described in the presence of media 199 containing penicillin and streptomycin. Epidermis is minced into 1 mm cubes and then placed into a metabolic shaker. Tritiated precursors and pharmacologic agents (when desired) are then added to the media. Incorporation is terminated at various time periods. Bovine keratohyalin is isolated by high-salt *in vitro* aggregation methods and specific activates are determined.

Major Findings:

1) The extractability of rat keratohyalin vs strength of phosphate buffer extractant has been clearly established and is comparable to bovine keratohyalin.

2) A protein reaggregate obtained following dialysis of a phosphate buffer extract of rat skin has been studied histochemically, ultrastructurally, and biochemically.

3) This reaggregate from rat skin is similar in many respects to bovine keratohyalin, but interesting ultrastructural and physical-chemical differences between bovine and rat keratohyalin have also been established.

4) Short term organ culture of bovine epidermis with subsequent chemical isolation of keratohyalin is feasible. Significant parameters - culture time, tissue/media rates, tritiated precursor amino acid/tissue ratio have been studied. Parameters were then idealized to make the organ culture system reproducible and statistically significant.

5) Bovine epidermis in organ culture will vary the synthesis (as monitored by specific activity) of bovine keratohyalin depending on the pharmacologic agent added to the media, but further work is needed to establish statistical significance.

Significance to Cancer Research:

The epidermis is an invaluable asset in studying carcinogenesis because of the epidermis' availability and its repid turnover of cells even under normal conditions. As an easily identifiable extractable protein keratohyalin can be used as a biochemical marker, and indeed, is absent in basal cell carcinoma, squamous cell carcinoma and psoriasis. Organ culture of bovine epidermis has the potential of being an effective screen to study both carcinogens and drugs.

Proposed Course of Project:

The project has been terminated.

Honors and Awards: None

Publications:

1) Klingler, W.G., Montague, P.M.: Extraction and Partial Characterization of Rat Keratohyalin Granules. Publication pending.

Serial No. NCI-3625
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Epidermal Macromolecular Synthesis in Cultured Embryonic Chick Skin and in Normal and Diseased (Benign and Malignant) Human Skin: A biochemical, Autoradiographical Study with Particular Emphasis on the Effects of Vitamin A.

Previous Serial Number: Same

Principal Investigators: Gary L. Peck, M.D., F. Ulrike Lichti, Ph.D.

Other Investigators: Vincent J. Hearing, Jr., Ph.D.

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total:	1.83
Professional:	0.93
Other:	0.90

Project Description:

Objectives:

1) To investigate cutaneous macromolecular biosynthesis; and to define the effect of vitamin A in inhibiting keratinization, in embryonic skin, normal adult skin, and in benign and malignant lesions.

2) To define certain dermatologic disorders at the biochemical level.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

1) 0.2-0.4 mm thick, Castroviejo keratotome slices of normal and diseased human skin. Skin from patients with Darier's Disease (NCI-3643) has been used to date.

2) 3-4 mm punch biopsies of normal and diseased human skin (NCI-3643.

3) Tarso-Metatarsal skin from 13-21 day chick embryos.

Methods Employed:

Chick embryo skin or skin from patients with Darier's Disease was incubated both with and without Vitamin A in a water bath shaker at 37°C for 1/2 to 6 hours in the presence of labelled precursors. Prior to exposure to radioactivity, chick skin was maintained in organ culture with and without vitamin A for 0-3 and occasionally up to 10 days.

After incubation, specimens were prepared for biochemical extraction and analysis. Various macromolecular fractions (DNA, RNA, protein, and glycoprotein) were isolated using standard methodology. The specific activities of these fractions were determined, and separation and characterization of individual molecular species was defined using polyacrylamide gel electrophoresis. Quantitative and qualitative changes in response to experimental design could be readily followed by banding patterns assayed both spectrophotometrically and by liquid scintillation counting.

Major Findings:

We have studied the effects of vitamin A on embryonic chick skin at the biochemical level. DNA synthesis as measured by ^{3}H-Thymidine incorporation declines progressively and becomes negligible by the 6th day after explantation. In contrast to other systems, vitamin A does not stimulate DNA synthesis. On the other hand, over all rate of protein and RNA synthesis is not affected by placing skin in culture. At the microscopic level, treatment with vitamin A causes mucous metaplasia, involving the inhibition of normal keratinization and the simultaneous stimulation of mucin production. Electrophoretic analysis indicates that synthesis of several major polypeptides is turned off by vitamin A administration (this would account for the inhibition of keratinization very nicely), but to date, we have been unable to find the stimulation of any protein which might represent the prosthetic group of mucin (a glycoprotein). On the other hand, when H^3-fucose (one of the sugars in epithelial mucins) is used as precursor, it can be seen, again with the aid of gel electrophoresis, that several glycoproteins are decreased in synthesis with vitamin A administration, while one low molecular weight glycoprotein (which is absent in normal tissue) is synthesized very actively. ^{35}S-sulfate incorporation into macromolecules of the epidermis, presumably sulfated micropolysaccharides, is also stimulated by vitamins. While further work is needed, these results would seem to indicate that vitamin A causes mucus metaplasia primarily by inhibiting keratinization, and at the same time allowing mucin formation to occur, presumably by the glycosylation of pre-existing protein in the chick epidermal cell.

Preliminary electrophoretic analyses have indicated that several major protein components found in normal skin are lacking in involved skin of Darier's. This data, when considered with the overall hypometabolism found in Darier's patients, might indicate that certain metabolic processes are inhibited in these patients. In response to vitamin A treatment, although the overall rate of protein synthesis was only slightly increased in Darier's skin, whatever metabolic block was functioning in the suppression of the several missing proteins, was effectively removed, and substantial synthesis of these proteins began in these tissues. It has been known for some time that occasional patients with Darier's Disease respond to topical and systemic therapy with vitamin A. This study may provide the first clue as to the mechanism of action of vitamin A in the treatment of Darier's Disease. The identity of these critical proteins remains to be established. We are currently studying the synthesis of the various RNA's in these tissues, and the response of their synthesis to vitamin A treatment, in an effort to determine at what level these inhibitory factors and the derepression mechanisms are located. These studies, it is hoped will indicate the metabolic abnormality in Darier's Disease, and hopefully, an effective way to correct it.

Our results indicate that vitamin A acts at the level of cellular control mechanisms, and, at least in the case of embryonic chick skin, normal and Darier's human skin, causes basic redifferentation of the cell at the molecular level.

Significance to Cancer Research:

Since carcinogenesis is an instance of altered differentiation, studies of vitamin A-induced reidfferentiation, both in human and chick skin, may serve as an excellent model for investigations of cellular control mechanisms which relate to carcinogenesis. The fact that carcinogen metabolism and carcinogenesis are influenced very markedly by vitamin A deficiency directly links research on vitamin A epithelial effects to cancer research.

Proposed Course of Project:

In chick skin, continued biochemical analysis will be done in order to more accurately characterize the epidermal mucin formed, and the mechanism by which vitamin A inhibits normal epidermal protein synthesis and stimulates mucin biosynthesis.

In Darier's Disease, more patients will be studied to confirm our preliminary observations, and to characterize the major epidermal proteins that are lacking in diseased skin. Skin from normal volunteers may be needed for comparative analysis.

Honors and Awards: None

Publications: None

Serial No. NCI-3630
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Vitamin A Acid Induced Mucous Metaplasia of Embryonic Chick Metatarsal Skin in Organ Culture: A Histochemical and Scanning and Transmission Electron Microscopic Study.

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Bruce K. Wetzel, Ph.D., Peter M. Elias, M.D., F. Ulrike Lichti, Ph.D., Stuart Yuspa, M.D.

Cooperating Units: Experimental Pathology Branch, DCCP

Man Years (July 1, 1973 through June 30, 1974)

Total:	0.74
Professional:	0.64
Other:	0.10

Project Description:

Objectives:

1) To investigate the mechanisms governing epidermal cell differentiation.

2) To morphologically investigate the mechanism by which vitamin A alters epidermal cell differentiation with resultant inhibition of keratinization.

3) To determine if epidermal cells are capable of both mucin and keratin production, or whether the two processes are mutually exclusive.

4) To study the early changes of vitamin A induced mucous metaplasia at the ultrastructural level.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

Metatarsal shank skin (devoid of feathers) is obtained from 13-14 day chick embryo.

Methods Employed:

Chick metatarsal skin is cultured in vitro and stimulated to undergo mucous metaplasia by vitamin A acid at varying doses. Specimens are taken at varying time intervals and evaluated for mucin production by the use of special stains, such as PAS (with and without diastase), Alcian Blue, Toluidine Blue, and Colloidal iron. Specimens are also fixed in glutaraldehyde for subsequent examinations under the transmission and scanning electron microscopes. In addition, the scanning electron microscope is being used to study in ovo skin scale and feather development, and their inhibition by vitamin A acid in vitro. The effect of retinyl acetate on the morphology of mouse epidermal cells in culture is also being studied.

Major Findings:

1) Using 30 international units of vitamin A acid per milliliter of culture media, it was found that mucous metaplasia occurred with 2 days in in vitro incubation. Maximum metaplasia took place by 6 days.

As little as 1 I.U. produced metaplasia in 6 days. Metaplasia was inhibited or reduced greatly at room temperature, or in the absence of serum. We have studied specimens of chick skin harvested prior to 2 days of incubation and treated with low dosage vitamin A in order to determine the earliest mechanism involved in mucus metaplasia. Ultrastructurally, we found no specific mechanism operative, other than increased amounts of rough endoplasmic reticulum and polyribosomes indicating increased protein synthetic activity.

2) Microscopically, the control specimen keratinized normally with the formation of thick bundles of tonofilaments, keratohyalin granules, and a multi-layered stratum corneum. Vitamin A acid treated specimens did not undergo keratinization. There was no tendency of the columnar basal cells to flatten as they approached the surface. Neither stratum corneum, nor keratohyalin granules were formed. Tonofilaments were present in small, fine bundles. In addition, there was increased folding of the nuclear membrane, and increased amounts of rough endoplasmic reticulum and polyribosomes. Mucin was present in the cytoplasm as membrane-bound electron dense material.

3) In addition to mucin formation there seems to be a glandular metaplasia in the epidermis with formation of acini. This morphologic change has not been demonstrated previously. Our standard system now utilizes 13-14 day old chick embryos treated with 20 i.u. vitamin A acid in Eagle's Minimal Essential Media with 5% fetal calf serum. Cultures are harvested at 2-3 days. 16 day and older chick embryo skin manifested a selective basal cell layer response to vitamin A.

4) Vitamin A induced mucus glandular metaplasia was inhibited by the addition of puromycin or cycloheximide to the culture medium; actinomycin D, hydroxyurea, and colchicine failed to inhibit this process. Thus, protein synthesis is necessary for metaplasia, while mitosis, and DNA and RNA synthesis are not.

5) Scanning electron microscopy revealed that metaplastic changes were visible as early as one day after vitamin A exposure. Individual epidermal cells lost attachments to their neighbors, became spherical, and developed surface villi. Selective basal cell layer effect was observed in 17 day chick embryos treated with vitamin A. Although the superficial stratified squamous layer of the epidermis was unresponsive to vitamin A, it was thrown into large folds by the hyperplastic masses of underlying metaplastic cells.

6) Chick skin scale formation is inhibited by vitamin A acid.

Significance to Cancer Research:

1) Vitamin A is known to inhibit carcinogenesis and to be of value in the treatment of malignancy. The mechanisms of action of vitamin A in inhibiting keratinization may be related to its antineoplastic activity.

Proposed Course of Project:

Continued ultrastructural examination of the above material.

Honors and Awards: None

Publications: None

Serial No. NCI-3631
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Treatment of Skin Cancer with Topical BCNU, and CCNU

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total:	.01
Professional:	.01
Other:	0

Project Description:

Objectives:

To evaluate the therapeutic effectiveness and toxicity of BCNU and CCNU administered topically to the skin surface of the above patients.

The Objective and Approach of the National Cancer Pland that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Agents:

0.1%, 0.2% and 0.4% Bis-Chloro-ethyl-nitrosourea; and 0.1%, 0.4% and 1.0% chloroethyl-nitrosourea; in 30% liquid petrolatum, 70% white petrolatum.

Material:

1) Basal Cell Carcinoma

2) Nevoid Basal Cell Carcinoma Syndrome

3) Xeroderma pigmentosum with cutaneous carcinoma

Methods Employed:

1) Patients must have pathologic confirmation of the clinical diagnosis.

2) They must have normal hematologic, hepatic, and renal function.

3) Lesions on eyelids and canthi are not included.

4) Patients are cautioned to avoid sun

5) Ointments are applied twice daily to affected areas for 8 weeks.

6) Laboratory tests are done before, during, and 6 weeks after therapy.

7) Serial photographs are taken.

Major Findings:

1) A total of 12 patients were included in this study. Nine received only BCNU ointment. One received only CCNU ointment; the remaining two received CCNU ointment initially and were given BCNU later as a second course of therapy. Five of the 12 patients were treatment failures by other modalities, including surgery and topical 5-FU in propylene glycol. Four of the patients had had X-ray therapy of their acne earlier in life. Four other patients had moderate to severe actinic skin. Two patients had the basal cell nevus syndrome; one had xeroderma pigmentosum. Most patients had lesions of the exposed areas of face and neck. Many patients also had lesions on the trunk and extremities. Nine of the 12 had the standard variety of BCE histologically; the remaining three were superficial BCE, morphea type of BCE, and the fibroepithelioma type of BCE (Pinkus). There were nine males, three females, varying in age from 23-65.

2) Results of treatement are as follows:

BCNU	0.1%	26 lesions	10 cures	15 partial responses	one no response
CCNU	1.0%	7 lesions	1 cure	5 partial responses	one no response

Partial response is defined as visible flattening of the tumor mass. The ten BCNU cures were in four patients.

3) To date, neither recurrences, nor signs of chronic toxicity have been observed.

4) Severe contact dermatitis has occurred in some patients with 0.1% BCNU ointment. The dermatitis appeared from day 2 to day 33 in the different patients. Several patients have not developed any cutaneous irritation. In three patients tumors disappeared without antecedent skin inflammation. Usually the patients who had no skin reaction to the medication had little to no therapeutic response. This supports the clinical impression derived from experience with topical 5-FU: No inflammation, No response.

5) The main difficulty in conducting this study was the lack of an assay system for the stability of the ointment. The active drugs are unstable in water or propylene glycol, therefore, petrolatum is the only suitable vehicle, and it is not possible to extract the active drug from the petrolatum ointment. This made establishing of the shelf-life difficult. By experience we determined that ointments over one month were not stable.

Significance to Cancer Research:

1) This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior agent to 5-Fluorouracil in propylene glycol which is widespread topical use.

2) We would also hope to contribute to the knowledge of the clinical pharmacology of this agent. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies.

Proposed Course of Project:

Concluded except for continued observation of test subjects to determine recurrence rate and for any signs of chronic toxicity.

Honors and Awards: None

Publications: None

Serial No. NCI-3632
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Toxic Epidermal Necrolysis and Graft-Versus-Host Reaction

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: P.M. Elias, M.D., G.P. Herzig, M.D., R.G. Graw, Jr., M.D.

Cooperating Units: Hematology Supportive Care Branch, NCI

Man Years (July 1, 1973 through June 30, 1974)

Total:	.05
Professional:	.05
Other:	0

Project Description:

Objectives:

To study Graft-Versus-Host Reaction (GVH) and Toxic Epidermal Necrolysis (TEN), and to investigate the relationship between them.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Methods Employed:

1) Taking of history, and repeated physical examination of a patient with acute lymphocytic leukemia, treated with allogeneic bone marrow transplantation, who developed GVH, and subsequently, TEN.

2) Review of literature

3) Examination with the electron microscope of multiple skin biopsies taken at various time intervals and at various skin sites.

Major Findings:

1) This is the first documented case in which the usual morbilliform eruption associated with sheet-like desquamation of the epidermis,

typical of TEN.

2) Early in the clinical course, cutaneous histopathology was typical for GVH.

3) Later in the clinical course, biopsies taken from intensely erythematous sites, near areas of massive epidermal desquamation showed more extensive epidermal destruction, consistent with the diagnosis of TEN.

4) Support for our thesis linking TEN in this patient with his GVH, comes from animal studies in which experimental GVH in hamsters and monkeys developed TEN. Furthermore, a case of GVH was found in the literature in which a bullous eruption, in retrospect compatible with TEN, developed.

5) Electron microscopic findings: In biopsies taken at distances greater than 10 cm. form the blister edge, ultrastructural changes were limited to the basal cell layer. The entire basal layer was involved in a uniform manner, while the spinous, granular, and horny layers appear virtually uninvolved. Mitochondrial swelling coarsening of tonofibrils, and increased prominence of ribosomes was noted in all basal cells. The nuclei appeared contracted, with more convoluted nuclear membranes; increased density of chromatin was noted. Biopsies closer to the blister edge displayed considerable intercellular edema, and occasional early bulla formation. Lymphocytes were occasionally seen within the basal layer, but only rarely above. Increased numbers of Odland bodies and abnormal keratohyalin granules were noted in the superficial epidermis. In summary, the initial pathologic events are in the basal layer, and it appears that the nomonuclear infiltrate may play a significant pathogenetic role.

Significance to Cancer Research:

1) Immunosuppressed patients, including treated cancer patients, who are transfused or transplanted with immunocompetent cells, may expect to develop GVH. In view of this case report, we may now expect some of them to develop TEN as well.

Proposed Course of Project:

Electron microscopic examination is continuing. Specimens from new patients with either TEN or GVH are also being studied.

Honors and Awards:

First Prize - 1971 North American Clinical Dermatological Society Memorial Essay Contest

Publications:

1) Peck, G.L., Herzig, G.P., and Elias, P.M.: Toxic Epidermal Necrolysis in a patient with Graft-Versus-Host Reaction. Arch. Derm. 105: 561-569, 1972.

2) Peck, G.L., Elias, P.M., Graw, Jr., R.G.: Graft-Versus-Host reaction and Toxic Epidermal Necrolysis. Lancet II (7792): 1151-1153, 1972.

3) Elias, P.M., Arndt, K.A., Peck, G.L.: Scalded Skin Syndrome in Adults. New Eng. J. Med. 288: 582-583, 1973.

Serial No. NCI-3633
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Treatment of Psoriasis with Topical CCNU. (IND-7003)

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Stephen Guss, M.D., and Douglas Key, M.D.

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total: .01
Professional: .01
Other: 0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical CCNU in psoriatic patients.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Agents:

0.4% and 1.0% CCNU in 30% liquid petrolatum and 70% white petrolatum.

Material:

Adult patients with psoriasis. Females must be past the childbearing age.

Methods Employed:

1) Patients must have psoriasis previously unresponsive to standard therapy.

2) They must have normal kidney, liver, and bone marrow function.

3) Medication will be applied twice daily until clear.

4) Patients will avoid sun exposure to prevent phototoxicity, if any.

5) Laboratory tests measuring bone marrow, liver, and kidney function will be obtained before, during, and 6 weeks after cessation of therapy.

6) Serial photographs will be obtained.

Major Findings:

1) Seven of 9 patients treated for longer than 2 weeks cleared completely. The 2 patients who did not clear had severe psoriasis of the palms and soles. Four additional patients were treated for less than 2 weeks.

2) Severe irritation of the skin surrounding the psoriatic plaques occurred. This was especially marked in the intertrigenous agreas.

3) Subsequent to the cutaneous irritation, persistent pigmentation, telangiectasia, and cutaneous pain developed as well as temporary bone marrow suppression.

4) Four patients have remained free of disease in CCNU treated areas since October, 1970 to the present.

5) No signs of delayed or chronic toxicity have appeared.

Significance to Cancer Research:

1) This study is part of an ongoing search for an effective topical therapy for psoriasis.

2) It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.

Proposed Course of Project:

Concluded except for continued observation of test subjects - for any chronic toxicity and for determination of length of remission.

Honors and Awards: None

Publications:

1) Peck, G.L., Guss, S.B. and Key, D.J.: Topical Lomustine in the Treatment of Psoriasis. Arch. Derm. 106: 172-176, 1972.

2) Peck, G.L., Guss, S.B. and Key, D.J.: Reply to Comment on Topical Lomustine Therapy for Psoriasis. Arch. Derm. 108: 273-274, 1973.

Serial No. NCI-3636
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Susceptibility of Fibroblasts from Xeroderma Pigmentosum Patients to Oncogenic Virus Transformation Post-irradiation in vitro.

Previous Serial Number: Same

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: Douglas Key, M.D. and Stuart Aaronson, M.D.

Cooperating Units: Viral Carcinogenesis Branch, NCI

Man Years (July 1, 1973 through June 30, 1974)

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives:

Xeroderma pigmentosum is a genodermatosis with a marked predisposition to development of carcinomas in sun exposed areas. A defect in DAN repair, post UV-damage, has been shown in most of these patients. The question arises as to whether the carcinogenesis shown in these patients is perhaps the expression of oncogenic virus activity initiated or allowed by the suspected enzyme defect in UV-DNA repair in these patients. The purpose of this study is to obtain parameters of both DNA and RNA oncogenic virus infection and transformation of fibroblasts from normals and patients with xeroderma pigmentosum after varying dosages of ultra-violet radiation.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.2: Delineate the nature and rate of oncogenic cell transformations in carcinogenesis (include aspects of cell culture, viruses).

Methods Employed:

Fibroblasts as grown out from skin biopsy explants to undergo SV-40, a DNA virus, transformation assay as described. Infectivity to be determined by immunofluorescent T antigen incidence; transformation by gross colony counts. Cell survival needed for obtaining the fraction of total colonies which are transformed will be determined by direct colony counts on feeder layers. Additional attempts will be made to perform parallel studies with RNA oncogenic focus forming viruses.

Major Findings:

With SV-40 virus infection post-irradiation of both normal and xeroderma fibroblasts there was no enhancement of transformation, but a decrease in transformation in proportion to the post-irradiation survival curve. The T antigen incidence, as a rough marker of infectivity was the same in irradiated as non-irradiated cells as measured 48 hours post infection.

Significance to Cancer Research:

The possible demonstration of enhanced susceptibility after UV irradiation to transformation by oncogenic viruses in human cells would be an exceedingly valuable *in vitro* model in view of: 1) the clinical predisposition to carcinogenesis in sun exposed areas in this rare genodermatosis; 2) possible relationship to predisposition of older populations to sun-induced cutaneous malignancies.

Proposed Course of Project:

Initial attempts to establish a RNA oncogenic focus forming virus infection in xeroderma cells proved unsuccessful. However, studies are now underway with the newly suspected primate agents. If xeroderma cells should prove susceptible to such an infection then parallel studies will be done as described.

Honors and Awards: None

Publications:

1) Key, D.J. and Todaro, G.J.: Xeroderma pigmentosum cell susceptibility to SV-40 virus transformation: Lack of effect of low dosage ultraviolet radiation and enhancing viral induced transformation. *Journal of Investigative Dermatology*. 62:7-10, 1974.

Serial No. NCI-3638
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of DNA repair in normal human cells and in cells from patients with xeroderma pigmentosum

Previous Serial Number: Same

Principal Investigator: Jay H. Robbins, M.D.

Other Investigators: Kenneth H. Kraemer, M.D., Alan D. Andrews, M.D., Evelyn Parker, Ph.D.

Cooperating Units: Hayden G. Coon, Ph.D., Laboratory of Cell Biology, DNCB, NCI; Rosemary Cuddy, Program Analysis and Evaluation Branch, DCG, NCI; Rufus S. Day, III, Ph.D., Chemistry Branch, DECP, NCI; Steven B. Zimmerman, Ph.D., Laboratory of Molecular Biology, NIAMD; David A. Newsome, M.D., Laboratory of Vision Research, NEI.

Man Years (July 1, 1973 through June 30, 1974)

Total:	4.9
Professional:	2.9
Other:	2.0

Project Description:

Objectives:

To study the DNA excisional repair process in normal cells and in cells from patients with xeroderma pigmentosum (XP). The role of this process in repair of damage caused by ultraviolet (UV) radiation, alkylating agents and other chemicals is being evaluated. The relationship between abnormalities in the function of this process and the development of skin and other tumors in humans is being investigated.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Materials:

Cells are obtained from hospitalized patients and outpatients. Cells studied include: XP and normal peripheral blood lymphocytes, dermal fibroblasts, conjunctival and corneal cells, epidermal cells, basal cell carcinoma cells and malignant melanoma cells. Lymphocytes from patients with chronic lymphocytic leukemia are also studied.

Methods Employed:

'Purified' lymphocytes are separated from other blood leukocytes by passage through nylon fiber columns. All other cells used are cultured by standard tissue culture methods. Fibroblast heterokaryons are formed by virus-induced fusion of cells from pairs of XP patients. The cells are cultured and exposed to UV light and then incubated with tritiated thymidine. DNA repair is assayed by measuring the UV-induced unscheduled thymidine incorporation by autoradiography and scintillation spectroscopy.

Major Findings:

1) We have evaluated the clinical manifestations and the DNA repair rates of our 16 XP patients who represent 13 kindreds. New clinical types of XP have been discovered among these patients. Our series of XP patients is the largest in which a valid attempt can be made to relate the various clinical manifestations of the disease to the patients' repair rates. Skin fibroblasts from all of these patients (and from related family members) are sent to the American Type Culture Collection for dissemination to other investigators.

2) Methods have been developed to study DNA repair in cultured conjunctival and corneal cells. It was found that such cells obtained from normal donors had greater rates of UV-induced DNA repair than similar cells from XP patients, thereby indicating that defective DNA repair may be the cause of at least some of the clinical abnormalities in these eye tissues of XP patients.

3) Genetic complementation studies are being performed in vitro in heterokaryons formed by fusing fibroblasts from pairs of XP patients. Thus, by combining two different XP cells it is possible to ascertain whether they have sufficiently different genotypes to permit restoration of the DNA repair process in the nuclei of the multinucleated heterokaryons. Two cell strains whose nuclei fail to show such restoration of the DNA repair process are said to be in the same complementation group and can be expected to have a genetic lesion affecting the identical gene product. Thus far, four complementation groups have been found among the patients whose cells we have studied, indicating the existence of at least four loci at which mutations can produce the XP repair defect. The four complementation groups were found to correlate with the magnitude of the repair defect (as reflected in the rate of UV-induced thymidine incorporation rather than with the clinical types of the disease e.g., presence or absence of neurological involvement). The recognition and identification of these complementation groups should be of considerable value in the biochemical investigations which are currently underway and

which are designed to recognize and purify the defective repair enzyme(s) in XP.

4) We have extended our complementation analysis of various lines of XP cells in collaboration with the laboratory of Prof. Dirk Bootsma, Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands. Prof. Bootsma's group had reported finding three complementation groups among their XP cell lines. Studies in each of our laboratories have found that two of our groups are different from any of their groups, while one of their groups was different from any of our groups. Thus, there appear to be at least five different XP complementation groups, suggesting that defects at any one of at least five genetic loci can give rise to XP.

5) Studies are being conducted in cooperation with Dr. Rufus Day, III, of the National Cancer Institute, to investigate the relationship between the rate of UV-induced radioactive thymidine incorporation (a measure of DNA repair) and the ability of the XP cells to repair (host-cell-reactivate) UV-irradiated adenovirus 2 and to form colonies after UV irradiation. The results indicate that cells from complementation group D, which have greater UV-induced thymidine incorporation than cells from groups A, B, and C, have less host-cell-reactivation and lower UV-survival than cells from these other groups, suggesting that the repair synthesis performed by cells in group D may differ qualitatively and not just quantitatively from that in normal cells or cells from other forms of XP.

6) Studies are being conducted to determine the reaction rate kinetics for UV-induced thymidine incorporation into normal donors' fibroblasts and into fibroblasts from XP patients in complementation groups C and D. An effort will be made to determine from the data obtained whether there are "fast" and "slow" components in the repair process and which of these may be defective in the XP cells from the various complementation groups.

7) We have been interested in the possible relationship between ageing and the ability to repair damage to DNA. In order to determine the effect, if any, of the number of cell divisions undergone in culture on the ability of cells to repair UV-induced damage to DNA, we are currently comparing rates of UV-induced DNA repair in the same cell line at different passage numbers for cells from most of our XP patients, some of their parents (heterozgotes), and normal donors. These cell lines have been carried through numerous cell passages for us by the American Type Culture Collection. Studies are currently underway to determine the effect, if any, of the cell passage number on the cells' rate of UV-induced DNA repair. We hope these studies will also cast some light on discrepancies between results reported in different laboratories using the same cell line at different passage numbers.

8) Attempts are being made to establish long term lymphoblastoid lines from XP patients' peripheral blood. The existence of such long term cultures would make possible several experiments not easily performed with fibroblast strains and would also make it easier to obtain large quantities of XP cells for biochemical studies designed to characterize

and purify the defective DNA repair enzymes in XP cells. Results to date indicate that the contamination of such cultures by mycoplasma make the cells totally unsuitable for UV-induced DNA repair studies. Therefore, methods have been employed to assure the development of lines free of mycoplasma infection. To date, such a mycoplasma-free lymphoblastoid line has been obtained from a normal donor which appears suitable for the study of the DNA repair process. Similar lines from XP patients are in the process of being established.

9) Studies have been performed on the effect of UV-radiation on chromosome number and structure in PHA-stimulated normal and XP peripheral blood lymphocyte cultures. The results indicate that UV-induced chromosomal abberations are many times more frequent in the XP cells than in the normal cells.

10) Studies are being performed with Dr. Steven Zimmerman to determine the levels of DNA ligase activity in fibroblasts from XP variants and from XP patients with decreased UV-induced DNA repair rates. Results to date indicate that these cells have normal levels of ligase activity.

11) Studies have been conducted on the rate of DNA repair in leukemia lymphocytes from patients with chronic lymphocytic leukemia. Our findings to date indicate that these cells have a normal rate of such repair in contrast to reports in the literature which claim these cells have markedly increased rates of repair.

Significance to Bio-medical Research and the Program of the Institute:

UV-radiation is believed to be the major cause of the most common type of human cancer, cancer of the skin. Patients with XP are particularly susceptible to the carcinogenic action of UV-radiation and develop multiple malignancies on sun-exposed areas of skin. Normal human cells have an excisional repair process which rapidly repairs DNA damage while most XP patients have a marked impairment in the rate of such repair. This process is involved not only in repair of UV-induced DNA damage but also in repair of damage by certain chemical carcinogens. Understanding the relationship between DNA repair deficiency and skin tumor development in XP patients would, therefore, elucidate the role of DNA repair in preventing in other humans those cancers which may be due to certain chemical and physical carcinogens.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Honors and Awards: None

Publications:

1) Robbins, J.H., Kraemer, K.H., Lutzner, M.A., Festoff, B.W., and Coon, H.G.: Xeroderma pigmentosum: An inherited disease with sun sensitivity, multiple cutaneous malignancies, and abnormal DNA repair. Ann. Int. Med. 80: 221-248, 1974.

Serial No. NCI-3643
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Wound Healing, Dermatome Stripping, and Dermabrasion Therapy in Darier's Disease (Keratosis Follicularis)

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: L. Dellon, M.D., P. Chretien, M.D., I. Cohen, M.D., W.G. Klingler, M.D., K. Kraemer, M.D., P. Elias, M.D., A. Andrews, M.D.

Cooperating Units: Surgery Branch, NCI

Man Years (July 1, 1973 through June 30, 1974)

Total:	.15
Professional:	.15
Other:	0

Project Description:

Objectives:

1) To observe the response of Darier's Disease to surgical therapy

2) To study the effect of experimental wounding on the clinical course of both involved and uninvolved skin.

3) To determine if the accelerated healing of dermatome stripped diseased skin in Darier's disease is due to an accelerated epidermal migration.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Material:

Normal and diseased skin in a patient with Darier's Disease.

Methods Employed:

1) Dermatome Stripping in patient 1. Strips of skin were taken from a 62-year-old female patient with Darier's disease using a Castroviejo ophthalmologic keratome (Storz Instrument Co.). Strips were approximately 2 cm. in width, 5 cm. in length and 0.2 mm. thick, determined by using a 0.2 mm. spacer in the shaving head. Sites included several areas of the back, upper abdomen and hips. Bleeding was controlled with pressure only. Telfa gauze dressings were used for one to two days, and no other topical therapy was applied. The wounds were then left open, measured and photographed daily.

Incisional Wound Healing. Multiple linear incisions, approximately one cm. in length, were made in diseased skin areas of the right anterior forearm and in clincally uninvolved skin of the left anterior forearm. Because the involved skin surface is more irregular than uninvolved skin, and because the epidermis is variably thickened in dermatoses such as Darier's disease, the standard blade guard device can not be used to ensure equal depth of incisions. We, therefore, chose minimal capillary bleeding as a measure of comparability of incision depth. Telfa gauze was the only dressing employed. Four mm. punch biopsies were taken at zero time, and 3, 6, 12, 18, 24, 48 and 72 hours after wounding. Each biopsy was divided transversely to the wound into two approximately equal pieces. One piece was fixed in cold phosphate-buffered glutaraldehyde, post-fixed in osmium tetroxide, imbedded in Epon 812, and 1-2 u thick sections were stained with Azure II-methylene blue. The other half was routinely fixed in formaldehyde and imbedded in paraffin prior to sectioning and staining. Sections were cut coronally to the incision.

2) Dermabrasion Therapy in patient 2. A 26 year old man with hypertrophic conifying Darier's disease especially severe on arms and legs was treated with surgical dermabrasion under general anesthesia several times. Rate of wound healing was measured by serial 6 mm punch biopsies of circular defects produced by 3 mm punches, and by serial punch biopsies taken from dermatome stripped sites of diseased and uninvolved skin punches.

3) Dermatome Stripping in patients 3 to 5. Two women and one man with severe, confluent, inflammatory, vesicular Darier's disease was treated with dermatome stripping of legs or buttocks under general anesthesia. Rate of wound healing was measured by the methods used in patient 2. One of these women also had dermabrasion of the face and excision of severely diseased labia majora.

Major Findings:

1) Dermatome Stripping in patient 1. Involved sties healed more rapidly than uninvolved skin following dermatome stripping. Two weeks after stripping, the uninvolved site remained larger and more erythematous than the involved site. Furthermore, a zone approximately 10 cm. wide

of previously diseased skin around stripped sites on the left hip was observed to have clinically regressed by this time. Clinical regression of involved skin surrounding dermatome stripped sites was repeatedly observed.

Five months after the initial stripping experiment, the patient had a generalized exacerbation of her disease, which spared previously dermatomed sites of uninvolved skin. Clinically, the uninvolved stripped sites are now hypopigmented and slightly atrophic. Histologically, the epidermis and pilosebaceous apparatus appear normal except for diminution of the rete ridge pattern and flattening of the papillary dermis.

Incisional Wound Healing. Superficial linear incisions healed more quickly in diseased sites (12 hours) than in uninvolved sites (48 hours) indicating accelerated epidermal migration.

2) Diseased sites healed more rapidly than uninvolved sites, using both methods of measuring rate of wound healing. Incisional wound healing studies were not done because of the difficulty in locating incisions in diseased tissue after 1-2 days. 3 mm defects in diseased skin healed in 2 d, and in uninvolved skin in 3 d. The patient responded well to dermabrasion and has less than 10% recurrence after 11 months of observation. Peripheral clearing, as seen in patient 1, was not observed.

3) Dermatome stripping was well tolerated in all patients producing long lasting (up to 30 months) disease free remissions. Peripheral clearing has occurred in 3 of 5 patients following dermatome stripping.

Significance to Cancer Research:

1) Knowledge of the controls governing cell migration after injury are essential to the understanding of malignant growth.

Proposed Course of Project:

1) Ultrastructural examination of the above material.

2) Confirmation of these findings in other patients with Darier's disease.

Honors and Awards: None

Publications:

Peck, G.L., and Elias, P.M.,: Wound Healing in a Patient with Darier's Disease: Preliminary Observations. In Maibach, H.I. and Rovee, D. (Eds.): Epidermal Wound Healing. Chicago, Ill., Year Book Medical Publishers, 1972, pp. 357-363.

Serial No. NCI-3644
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Establishment of a Screening System for Topical Chemotherapeutic Agents in the Therapy of Skin Cancer, Mycosis Fungoides, and Psoriasis

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Stephen K. Carter, M.D.

Cooperating Units: Division of Cancer Treatment, Cancer Therapy Evaluation

Man Years (July 1, 1973 through June 30, 1974)

Total:	.10
Professional:	.10
Other:	0

Project Description:

Objectives:

The establishment of a scientific program in which present and future chemotherapeutic agents may be screened topically for antimitotic potential, toxicity, and possible therapeutic use initially in malignant skin diseases, such as cutaneous carcinoma and mycosis fungoides, and subsequently in benign hyperplastic diseases such as psoriasis.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.2: Discover new therapeutic agents and develop means for their selection.

Patients Needed:

At least 10 adult men or women for each chemotherapeutic agent tested topically in the treatment of skin cancer, mycosis fungoides or psoriasis. Females must be past the childbearing age.

Methods Employed:

After chemotherapeutic agents have been supplied by the Division of Cancer Treatment, NCI, and after these agents have been screened for biological activity and safety in experimental models, then large scale clinical testing will be done in mycosis fungoides, psoriasis, and skin cancer. It is anticipated that the initial steps in this project will be patch test screening for biological activity in patients with psoriasis and mycosis fungoides and percutaneous absorption studies with radiolabelled chemotherapeutic agents in laboratory animals.

The clinical protocols of this large scale clinical testing have not been formulated as yet. However, the basic design will be similar to NCI-3631 and NCI-3633.

Major Findings :

In progress

Significance to Cancer Research:

1) This program is a national cooperative study designed to discover superior topical chemotherapeutic agents than those which are currently available in the treatment of skin cancer, mycosis fungoides, and psoriasis.

2) We would also hope to contribute to the knowledge of the clinical pharmacology of these agents. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which allow for long term studies.

Proposed Course of Project:

The entire design of this screening system, including preclinical screens and large scale clinical testing, is currently being established with the assistance of a committee of dermatologists, pharmacologists, toxicologists, and chemotherapists. This program will complement an Arthritis Institute program for psoriasis.

Honors and Awards: None

Publications: None

Serial No. NCI-3647
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The fine surface morphology, ultrastructure, and cytochemistry of normal and diseased leukocytes during development, in the circulation, and in sites of leukocytic infiltrates.

Previous Serial Number: Same

Principal Investigator: Bruce Wetzel, Ph.D.

Other Investigators: William R. Levis, M.D. and Elaine B. Alexander

Cooperating Units: Immunology Branch, NCI

Man Years (July 1, 1973 through June 30, 1974)

Total:	2.5
Professional:	0.5
Other:	2.0

Project Description:

Objectives:

To define the surface ultrastructure of leukocytes under a wide range of conditions and thereby provide fresh insight into the physiology of normal and leukemic cells; also, to characterize definiteively the origin, contents, and fate of the characteristic granules of neutrophils, eosinophilis, and basophils as an index of the development, function and fate of these cells.

The Objective and Approach of the National Cancer Plant that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

Whole blood was introduced directly from venipuncture into a ten-fold volume of agitated aldehyde fixative warmed to 37°C. After fixation for 90 minutes the white cells were concentrated by differential sedimentation. Aliquots of white cells concentrated from anticoagulated blood in buffy coats and by the Ficoll-Hypaque procedure were fixed in suspension for SEM, and parallel Aliquots were incubated on nylon fiber columns;

non-adherent leukocyte populations were then collected from the column and fixed in suspension for SEM. Leukocytes fixed in suspension were deposited, stained, and washed on Nuclepore filters, trapped in a thin film of water, quick frozen, and freeze-dried for SEM. Individual cells examined by SEM could be relocated by light microscopy (LM) and photomicrographed for positive identification of cell type. Some additional specimens have been prepared for transmission electron microscopy (TEM) with a variety of fixatives.

Major Findings:

Leukocytes fixed directly in whole blood display distinctive surface features which apparently are altered by exposure to anticoagulants and sedimentation procedures carried out on live cells. Monocytes are generally covered with microvilli and resemble large lymphocytes more than the characteristically ruffled monocytes seen in other preparations. Lymphocytes fixed in suspension in whole blood, and throughout the nylon column separation procedure are uniformly covered with microvilli. We suspect that the smooth-surfaced forms described by others (as distinctive of "T" lymphocytes) are artifactual. Adherent and nonadherent lymphocyte populations from the nylon columns appear to be indistinguishable by SEM, although they appear to represent predominantly "B" lymphocytes and nearly pure "T" lymphocytes, respectively, based on immunofluorescent staining for surface immunoglobulin on cells which fail to ingest latex beads.

Significance to Cancer Research:

SEM study of buffy coat cells in culture offers a novel and informative view of these cells undergoind attachment, motion, interaction, phagocytosis, response to physical and chemical change, blastogenesis, and degeneration, and provides a new basis for comparison with leukemic cells. The key roles of lymphocytes and macrophages in immune responses to malignant growth and therapy are largely dependent on cell surface phenomena and cell interaction - both uniquely amenable to study by SEM and ancillary methods.

The characteristic granules of neutrophils, eosiniphils and basophils are the principal indices of differentiation and the principal effector organelles of these cells; a thorough understanding of the origin and nature of these granules is necessary to define and interpret the differentiation of normal granulocytes, the cytopathology of chronic granulocytic leukemia and related disorders, and to help clarify the nature and functions of granulocytic infiltrates in skin and other tissues in malignant diseases.

Proposed Course of Project:

Our success in developing methods for the SEM study of positively identified leukocytes, and out initial experience with those from the peripheral blood of normal individuals will be applied in studies of normal development in the hematopoietic tissues, exudates and culture systems, and especially in a survey of leukemic conditions. A major study of leukocyte granulogenesis is planned employing quantitative autoradiography, cytochemistry and TEM. Light microscopic monitoring of preparative conditions and the dynamics of leukocyte adherence, motility, interaction and growth are planned with the use of thin perfusion chambers.

Honors and Awards: None

Publications: None

Serial No. NCI-3648
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: T Cell Leukemias and Lymphomas: Morphologic and Functional Studies of the Neoplastic Cells

Previous Serial Numbers: NCI-3648 and NCI-3653

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: Richard L. Edelson, M.D., Allan D. Andrews, M.D.

Cooperating Units: Philip Schein, M.D., Medicine Branch, NCI, Ira Green, M.D., and Charles Kirkpatrick, M.D., National Institute of Allergy and Infectious Disease; Tab Ahmed and Michael Facktor, U.S. Naval Hospital.

Man Years (July 1, 1973 through June 30, 1974)

Total:	2.0
Professional:	2.0
Other:	0

Project Description:

Objectives:

1) To determine whether those neoplastic cells which preferentially infiltrate the skin share common features.

2) To relate the clinical findings in these patients to the distinctive properties of the neoplastic cells.

3) To establish more effective means of therapy of these disorders through exploitation of the distinctive properties of the neoplastic cells.

4) To use the homogeneous cells found in these disorders as reagents for investigation of T cell function.

5) To isolate the product of the mixed leukocyte reaction locus.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

1) Tissues to be studied are sectioned in a cryostat, layered with sheep erythrocytes containing on their surfaces moieties which will specifically interact with receptors on mononuclear cells, rinsed to remove nonadherent cells, fixed in glutaraldehyde, and stained with H and E to be viewed by light microscopy.

2) Tissue, cells and buffy coat preparations are Feulgen-stained and examined with cytophotometer in order to detect presence of polyploid cells.

3) Lymphocytes are grown in short term culture and the karyotype determined.

4) Lymphocytes and tissue were fixed and stained routinely for electron microscopy.

5) Lymphocytes are tested for sheep erythrocyte receptors, complement receptors, Fc receptors, IgG receptors, surface immunoglobulin, and specific antigenic determinants.

6) Lymphocytes are tested for responsiveness to the mitogens phytohemagglutinin, pokeweed and concanavalin A and for stimulating and responding capabilities in mixed leukocyte cultures.

7) Lymphocytes, fibroblasts and keratinocytes are tested for effector and target cell potential in cell mediated lympholysis reaction.

8) An antiserum specific for T cells has been produced and purified by absorption with B cells, red cells, and platelets. This antiserum may be used therapeutically in cases unresponsive to other more conservative measures.

9) Leukapheresis, a process by which blood is passed through a continuous flow centrifuge with white cells being selectively removed from the buffy coat, has been used in the therapy of one patient.

Major Findings:

1) T cells, of at least certain morphologic varieties, preferentially infiltrate the skin and spare the bone marrow.

2) If the Sezary syndrome is redifined as T cell leukemia with associated erythroderma, we have now identified three morphologic variants.

3) Each patient in this expanded Sezary group differs from the other patients at the cellular level, using functional, antigenic and morphologic criteria. In addition, several patients had unique clinical features, i.e., Nathan's inverse cyclicity and Stern's recurrent hepatic and renal infiltration.

4) The first case of lymphocytic leukemia with both B and T cell markers on the same cells was identified.

5) Review of the cumulative NIH experience with the Sezary syndrome revealed a remarkable lack of thymic infiltration at autopsy. This plus the identification of T cells in the skin of our more recent patients suggests that the skin may actually be playing a role in the aberrant differentiation of these abnormal cells.

6) An affinity of T cells for the skin is supported by our observation that the large majority of cells in mycosis fungoides, lichen planus, Mucha Habermann's disease, parapsoriasis en plaque and lymphomatoid papulosis also appear to be T cells.

7) One patient with T cell leukemia and tumor stage mycosis fungoides superimposed on a generalized erythroderma was treated with leukapheresis, a process by which leukocytes are selectively removed from the buffy coat in a continuous flow centrifuge, and the cutaneous lesions resolved. The patient returned 100 days later from California for a repeat of the leukapheresis with similar results.

8) Cells from one patient with the Sezary syndrome have been maintained in culture for one month and retain both their receptors for sheep erythrocytes and their distinctive morphology.

9) An immunotherapy protocol has been devised and accepted for use. However, because of the relative success of our more conservative therapeutic approaches, immunotherapy has not yet been required. Other approaches include the present trial run of L-asparaginase which appears to be of at least limited efficacy.

Significance to Cancer Research:

By identifying the roles of mononuclear cells in defense against non-lymphoid cancer and accurately characterizing the types of lymphoid cells causing various lymphomas, a more rational approach to the treatment and prevention of these diseases is possible.

Proposed Course of Project:

We hope to characterize a broad spectrum of cutaneous disease so that the underlying pathologic processes will be better understood. This work will involve the study both of patients and experimental diseases.

Honors and Awards: None

Publications:

1) Edelson R., Smith R., Frank M., and Green I.: Identification of subpopulations of mononuclear cells in cutaneous infiltrates. J. Invest. Dermatol. 61: 82-89, 1973.

2) Shevach, E.M., Edelson R.L., Frank M., Lutzner, M.A., and Green I.: A human leukemia cell with both B and T cell surface receptors. Proc. Natl. Acad. Sci. 71: 863-866, 1974.

3) Smith R.W., Blaese, R.M., Hathcock K.S., Buell D.N., Edelson R.L., and Lutzner M.A.: T and B lymphocyte markers in lymphoid cell research and in human diseases. Eighth Leucocyte Culture Conference. (in press)

4) Edelson R.L., Kirkpatrick C.H., Shevach E.M., Schein P.S., Smith R.W., Green I., and Lutzner M.A.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes: Morphologic and functional studies; Ann. Intern. Med. (in press) June, 1974.

Serial No. NCI-3649
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Enzymatic Control Mechanisms in Melanogenesis

Previous Serial Number: Same

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total:	1.6
Professional:	0.8
Other:	0.8

Project Description:

Objectives:

To investigate the control mechanisms involved in regulating melanogenesis in a variety of normal and malignant tissues.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Tyrosinase and other enzymic activities are investigated both by spectrophotometric and radioactive assay methodology. Tissues are dissected immediately before use in 0.1 M phosphate buffer, homogenized and fractionated into subcellular fractions by means of density gradient centrifugation and differential centrifugation. Samples are then incubated with the appropriate substrates and controls. The production of melanin and other reaction products can be followed spectrophotometrically or by liquid scintillation counting of the newly formed radioactive melanin.

Major Findings:

Since only one enzyme (tyrosinase) is essential for melanin biosynthesis, it is a unique system for the study of ensymatic control mechanisms in normal and malignant tissues. We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, but not peroxidase, as has been suggested by some workers. It has always been a subject of dispute how tyrosinase, which can be easily demonstrated to be present in an active configuration in the endoplasmic reticulum and Golgi apparatus, is inactivated in vivo in mammalian systems and subsequently activated once in situ in the melanosome. In amphibians, the mechanism seems to be proteolytic activation; in mammalian and avian systems however, proteolysis has no function in this regard. It has been found that tyrosinase is under allosteric control and that phospholipases play a part in the expression of the enzyme's activity. It has also been found that this allosteric regulation in murine melanomas is altered; this perhaps explains the atypical melanosome formation in these tissues.

Significance to Cancer Research:

In view of the differences noted between the regulation of tyrosinase in normal and melanoma tissues, we feel that at least one of the primary differences between the melanogenic capabilities of these two tissues has been revealed. The fact that these controls are operational at the post-translational level is informative; whether other levels of cellular control mechanisms are affected by carcinogenesis remains to be investigated.

Proposed Course of Project:

Differences in the control mechanisms over melanogenesis in normal and malignant melanoma tissues will continue to be investigated. The characteristics of enzymic activity and control in various subcellular fractions are being studied. This project has been expanded to include characterization of similar metabolisms in normal and malignant human tissues.

Honors and Awards: None

Publications:

Hearing, V.J.: Mammalian melanogenesis tyrosinase versus peroxidase involvement, and activation mechanisms. Arch Biochem Biophys 158:720-725, 1973.

Hearing, V.J.: Tyrosinase activity in subcellular fractions of black and albino mice. Nature 245:81-83, 1973.

Serial No. NCI-3650
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemical Characterization of Mammalian Melanosomes

Previous Serial Number: Same

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: Walter G. Klingler, M.D.

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description:

Objectives:

To characterize the structural and enzymatic composition of melanin granules throughout their development in vivo, both in normal and abnormal pigmentary systems.

The objective of the National Cancer Plan that this project most closely supports is 3.1: To study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Methods Employed:

Our research has been aimed primarily at characterizing the chemical composition of melanosomes in the mouse, both in the normal black mouse as well as in melanoma tissue. Eyes are excised and homogenized in 0.25M sucrose, and the melanosomes are then isolated and purified by means of an extensive series of differential centrifugations. Granules thus isolated are solubilized in either sodium decyl sulfate, 8 M urea or Triton X-100, then examined with the aid of polyacrylamide gel electrophoresis. Computer analysis of the resulting protein separations allows the calculation of various physio-chemical parameters of these macromolecules, including molecular weight, molecular size, valence and several other physical constants that are most useful in the comparisons of various proteins.

Major Findings:

The melanosome in normal mouse tissue is composed of multiple proteins, many of which are loosely bound and easily extracted. These probably constitute the proteins of the limiting membrane of the organelle. Another protein is of lower molecular weight and is tightly bound to the granule, and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. In addition, this latter protein seems likely to be tyrosinase (if this is the case, tyrosinase would have both structural and enzymatic properties, an unusual occurence in biological systems). Melanosomal proteins from melanoma tissues vary from those of normal tissue. Several of the proteins seem to be missing completely from these granules, which are also structurally distinguishable from normal granules by electron microscopy. Perhaps more importantly, many of the proteins in melanoma melanosomes are unique and are not found in normal melanin granules.

Significance to Cancer Research:

The observations concerning the aberrant biochemical characteristics of the melanoma melanosome indicate that the control of melanogenesis in malignant melanoma is in some manner affected by carcinogenesis. Melanomas are unusual among cancers since they do not result in complete differentiation of the affected melanocyte, but allow this cell's specialization, i.e. melanogenesis, to continue. These results indicate that although melanogenesis takes place, its metabolic pattern is abnormal.

Proposed Course of Project:

Since there are indications that the metabolism of melanogenesis in murine melanoma is aberrant, our research is ultimately intended to examine human melanomas, and (assuming melanosome formation is also affected there) attempt to characterize the level on which these disorienting control mechanisms operate. It is hoped that further insight into such controls, be they at the level of replication, transcription, translation, or post-translation, will provide clues as to the level on which carcinogenic information is expressed. In addition, the aberrant proteins found in the malignant tissue will hopefully be taken advantage of for the immunotherapy of malignant melanoma.

Honors and Awards: None

Publications:

Hearing, V.J. and Lutzner, M.A.: Mammalian melanosomes: characterization by polyacrylamide gel electrophoresis, Yale J. Biol. Med.,46:553-559, 1973.

Hearing, V.J. and Eppig, J.J., Jr.: Biochemical aspects of melanogenesis in normal and murine melanoma tissue. J. Cell Biol 59:137a, 1973.(Abstract)

Serial No. NCI-3651
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Melanogenesis and Immune Response in Human Melanoma

Previous Serial Number: None

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: Richard Edelson, M.D.
Richard Smith, M.D.

Cooperating Units: NCI, Immunology Branch

Man Years (July 1, 1973 through June 30, 1974)

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives:

1. To characterize melanogenic capabilities of human melanomas and nevi.

2. To culture melanocytes from human melanomas as target cells for immune response studies.

Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Methods Employed:

1. Tissues selected for examination are dissected out and fixed immediately in glutaraldehyde, then osmium, followed by dehydration, embedding, sectioning, and staining by conventional procedures. Histochemical staining for tissues is also carried out by published electron microscopic techniques.

2. Histiocytes and lymphocytes are identified on the basis of surface receptor sites both in paraffin sections and in culture, allowing the differentiation between B cells, T cells and histiocytes. Samples dissociated by trypsin are then separated from the noncellular com-

ponents by filtration and the intact cells then plated out and cultured on RPMI 1640 culture medium with 10% fetal calf serum.

Major Findings:

Nevus cells have been successfully cultured for the first time. These cells contained specific differentiation antigens. Superficial malignant malanomas are infiltrated by macrophages and T cells, while a large congenital nevus was found to be infiltrated by B cells only, and a deep melanoma contained neither type of cell. EM histochemistry demonstrated that both the superficial (pigmented) and the deep (nonpigmented) melanoma tissue contained tyrosinase, the enzyme responsible for melanin formation, but that in the deep tumor this enzyme seems to be supressed from acting in vivo.

Significance to Cancer Research:

It is important to culture melanoma cells so that properly controlled experiments can be carried out regarding their abnormal metabolisms. One such obvious difference is the different degrees of melanogenesis expressed in these tissues; another is the varying surface antigenic properties of these cells.

Proposed Course of Project:

Although the study is just underway, we are preparing to establish cultures of both melanotic and amelanotic melanomas; to characterize their aberrant melanogenic capabilities (in particular to determine the method of tyrosinase inhibition functioning in the amelanotic strain). In addition, we are most interested in pursuing research aimed at determining the unique antigenic properties of the melanocytes, which might be critical to immunotherapy research on malignant melanoma.

Honors and Awards: None

Publications: None

Serial No. NCI-3652
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Mechanisms of Cellular Immunity in Man

Previous Serial Number: NCI-3652 and NCI-3641

Principal Investigator: William R. Levis, M.D.

Other Investigators: John A. Powell, M.D., Samantha Davi - 12/73 - 5/74

Man Years (July 1, 1973 through June 30, 1974)

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives:

1) To investigate the mechanisms concerned with the control of mitosis, differentiation, function, and potentialities of normal and pathologic cells, with particular reference to the lymphocyte and cells of the lymphoreticular system.

2) To investigate the functional and morphological interactions between lymphocytes and macrophages and among different populations of lymphocytes during *in vitro* immune responses related to delayed hypersensitivity, homograft reactions, and cell mediated allergic contact dermatitis.

3) To detect, characterize, and isolate biologically active substances, including soluble mediators of cellular immunity, produced in stimulated and unstimulated human leukocyte cultures. To study their effects on supporting, augmenting, and inhibiting blastogenesis, cell division, modulation, and differentiation *in vitro*.

4) To develop and *in vitro* system for studying allergic contact dermatitis in humans including a method of monitoring immunotherapy of skin cancers with DNCB (see NCI-3641).

5) To develop a useful and sensitive histocompatibility test in humans.

6) To develop an in vitro model for the study of homograft rejection in humans.

7) To develop an in vitro system for studying immune reactivity to various skin cancers.

8) To determine the efficacy of 2, 4 dinitrochlorobenzene (DNCB) on cutaneous malignancies and premalignant growths in subjects sensitized to DNCB.

9) To evaluate DNCB as a useful model for the study of immunotherapy of certain human cancers and possibly lead to additional immunotherapy studies.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Material:

1) Lymphocytes and other peripheral blood cells are obtained from hospitalized patients and outpatients.

2) Keratatome strips of human skin.

3) Excised tumors of human skin.

Methods Employed:

1) Human peripheral blood lymphocytes are cultured in vitro and stimulated to transform into large blastoid cells by factors such as phytohemagglutinin, antigens, and foreign tissue. This phenomenon of blastogenesis is evaluated by the morphologic and biochemical changes which the lymphocytes undergo. The mechanisms by which these blastogenic factors act and the culture conditions required for blastogenesis are determined.

2) Human peripheral blood leukocytes are separated into a glass-adherent cell population, composed largely of macrophages, and into a population consisting of small lymphocytes. By recombining such populations obtained from the same and from different individuals, the function of each population is determined. Interactions and contacts between cells are examined by light and scanning electron microscopic techniques, including methods for observing the same cells by both modalities.

3) A method has been developed for freeze storing leukocytes for subsequent studies of the blastogenic response to various antigens and mitogens.

4) A method has been developed for adapting the dinitrochlorobenzene contact sensitization test to the *in vitro* lymphocyte transformation system.

Major Findings:

1) Methods for obtaining 'purified' lymphocytes, glass-adherent mononuclear cells, and a population containing both cell types from human peripheral blood have been developed and analyzed. We have improved and utilized a very rapid and simple 5-minute centrifugation method using heparinized whole blood for obtaining a population of mononuclear leukocytes (lymphocytes and monocytes) with very few contaminating granulocytes. This mononuclear leukocyte population contains the two cell types required for the blastogenic response and provides cultures capable of responding with maximal responses to sepcific and nonsepcific blastogenic factors. Parameters affecting the millipore filter assay technique for measuring tritiated thymidine incorporation into DNA in leukocyte cultures have been studied and analyzed.

2) Human epidermal cells induce balstogenesis in allogeneic leukocytes. Human epidermal cells did not induce blastogenesis in autologous leukocytes nor in the leukocytes of a mixed leukocyte HL-A identical sibling pair. These results indicate that epidermal induced leukocyte blastogenesis is a measure of histocompatibility in humans comparable to a one-way mixed leukocyte reaction.

3) A method has been developed which permits positive light microscopic identification of individual leukocytes in a heterogeneous population by Giemsa staining while preserving delicate surface features for subsequent scanning electron microscopy of the same cells. (see NCI-3647).

4) Isolation and partial characterization of a factor released by glass adherent phagocytic cells which is an essential requirement for the blastogenic response of lymphocytes to specific antigens, leukocytes, and phytohemagglutinin.

5) Isolation of a factor released in clutures containing lymphocytes of subjects sensitized to DNCB, that will induce balstogenesis in leukocyte cultures from nonsensitized subjects.

6) Characterization of the abnormal blastogenic responses of lymphocytes from patients with chronic lymphocytic leukemia.

7) Development of a method for monitoring the degree of sensitivity to DNCB *in vitro* during *in vivo* contact sensitization. This *in vitro* technique expands the usefulness of DNCB for the evaluation and study of cell mediated immunity, and offers a method for monitoring DNCB immunotherapy, an approach for the study of tolerance, and a controlled system for the study of the many antigen-released mediators of cellular immunity in man. Similar approaches with other contact allergens may offer a safe diagnostic test for allergic contact dermatitis.

8) Successful adaptation of the Dinitrochlorobenzene (DNCB) contact sensitization test to the *in vitro* lymphocyte transformation technique in humans.

9) Development of simplified methods for freeze storing human peripheral blood leukocytes.

10) Using the frozen leukocyte technology and the method for studying DNCB sensitivity *in vitro* we have demonstrated a temporal relationship between *in vivo* skin reactivity and *in vitro* specific lymphocyte transformation during a primary immune response.

11) Peak *in vitro* activity was observed two to four weeks following application of the primary sensitizing dose of DNCB and fell off to lower levels within two months of primary sensitization. These sequential studies of allergic contact dermatitis in humans are the first such studies of the kinetics of a primary cellular immune response using both *in vivo* and *in vitro* techniques.

12) Additional studies have demonstrated a quantitative relationship between the intensity and duration of allergic contact dermatitis to DNCB *in vivo* and the magnitude of specific lymphocyte transformation *in vitro*.

13) Demonstration that repeated challange with DNCB boosts specific *in vitro* lymphocyte transformation.

14) Discovery of lymphokine activity in allergic contact dermatitis there is evidence that such lymphokines may be the mediators of cell mediated immunity.

15) Demonstration of the time course of release of lymphokine activity in human peripheral blood cultures.

16) Demonstration of reproducible dose-response curves for lymphokine activity. Such dose-response curves illustrate a sensitive quantitative assay for cellular immunity *in vitro* and offers a biological system with great potential for further characterizing and isolating the mediators in cell mediated immunity.

17) The DNCB system offered the versatility that made it possible to show the lymphokines responsible for lymphocyte transformation in target leukocyte populations from nonsensitized subjects were as active on syngeneic (identical twins) leukocytes as on allogeneic leukocytes, indicating the observed activity was not due to histocompatibility factors.

18) It has recently been shown that lymphokine activity is absent during a primary immune response to DNCB and requires one or more rechallanges before activity is detectable using the lymphocyte transformation system which suggests the soluble factors known as lymphokines, may represent an amplification of cellular immunity.

19) Preliminary data on the characterization of these DNCB-induced lymphokines indicate they are lyophilizable, heat labile, stable under several cycles of freeze thawing and in polyacrylamide gel electrophoresis elute out in the region of albumin with 30% recovery pH 9.1.

20) Successful adaptation of an automated microsystem for harvesting cells had enabled us to harvest cells in a rapid fashion.

21) The unique features of this project are: a) Both *in vitro* and *in vivo* portions of this project are ethically feasible in humans which makes these studies more germane to human disease. b) Previous studies with microbial antigens have lacked effective control over exposure, in contrast to controlled DNCB snesitization. c) The assay is sensitive, specific and quantitative making it a valuable tool for measuring lymphocyte activation under a variety of experimental conditions.

Significance to Bio-Medical Research and the Program of the Institute:

The lymphocyte is a cell involved in immune reactions which may be operative in the immune response to neoplastic tissue. Our studies concerned with blastogenesis and the control of cell didivion and differentiation of the lymphocyte may elucidate this cell's normal and abnormal functions in the immune response involved in the host's defense against cancer. These studies should also provide information concerning the control of cell differentiation and division.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Honors and Awards: None

Publications:

1) Miller, A.E., and Levis, W.R.: Lymphocyte transformation during DNCB contact sensitization: An *in vitro* - *in vivo* evaluation of the primary immune response in man. *J. Clin. Invest.* 52: 1925-1930, 1973.

2) Wetzel, B., Erickson, B.W., and Levis, W.R.: The need for positive identification of leukocytes examined by scanning electron microscopy. Scanning Electron Microscopy. O. Jahari and I. Corvin (Eds.). ITT Research Institute, Chicago, Illinoid, 535-542, 1973.

3) Levis, W.R., Whalen, J.J., and Miller, A.E.: Lymphokine production in cell mediated allergic contact dermatitis. *Lancet* 2: 389-390, 1973.

4) Miller, A.E., Jr., and Levis, W.R.: Studies on the contact

sensitization of man with simple chemicals: I. Specific lymphocyte transformation in response to Dinitrochlorobenzene sensitization. J. Invest. Dermatol. 61: 261-269, 1973.

5) Levis, W.R., Whalen J. J., and Miller, A.E., Jr.: Studies on the contact sensitization of man with simple chemicals: II. Lymphokine production in allergic contact dermatitis to Dinitrochlorobenzene. J. Invest. Dermatol. 62: 2-6, 1973.

6) Levis, W.R., Kraemer, K.H., Klingler, W.G., Peck, G.L., and Terry, W.D.: Topical immunotherapy of basal cell carcinomas with Dinitrochlorobenzene. Cancer Res. 33: 3036-3042, 1973.

7) Levis, W.R., Whalen, J.J., and Miller, A.E., Jr.: Blastogenesis of autologous, allogeneic, and syngeneic (Identical Twins) lymphocytes in response to lymphokines generated in Dinitrochlorobenzene sensitive human leukocyte cultures. J. Immunol (In press).

Serial No. NCI-3654
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Development and Application of Approaches and Methods of Scanning Electron Microscopy (SEM) in Studies of Soft Biological Tissues.

Previous Serial Number: Same

Principal Investigator: Bruce Wetzel, Ph.D.

Other Investigators: Grace B. Cannon, Ph.D., and Elaine B. Alexander, M.D.

Cooperating Units: Litton Bionetics, Bethesda, Md., and Immunology Branch, NCI.

Man Years (July 1, 1973 through June 30, 1974)

Total:	2.5
Professional:	0.5
Other:	2.0

Project Description:

Objectives:

To discover and develop new methods and interpretive criteria for the application of SEM to a wide variety of soft biological tissues, and thereby capitalize on the unique capabilities of this instrument in providing 1) improved survey and sampling as an adjunct to other studies, 2) an unparalleled three-dimensional, experimental view of cell form and surface phenomena, and 3) an unusual potential for cytochemical studies.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.5: Study cell surface and cell membranes.

Methods Employed:

A variety of tissue culture cells which had been grown on coverslips were fixed *in situ*, stained, mapped, and photographed without drying by microscopy and then processed appropriately for SEM. In addition, a new procedure was developed to visualize by both SEM and light microscopy (LM) non attached cells fixed in suspension and recovered in high yield. The fixed cells were applied to Nuclepore filters, stained and washed, trapped in a thin film of water on top of the filters, frozen quickly, freeze-dried, and coated with evaporated carbon and gold/palladium. After scanning individual cells could be relocated and examined by light microscopy.

Major Findings:

Methods which enable scanning microscopy of cells previously stained, identified, and mapped by light microscopy, have proven useful in studies of a wide variety of cells in tissue culture, (chick embryo) cells, fibroblast lines, melanomas, fibrosarcomas, myosarcomas, renal carcinomas, and cells from pleural effusions of breast cancer patients. Distinctive surface morphology has been found in a few cell lines, but considerable variation has characterized most cultures. Some of this morphological variation is due to asynchronous cell division as cells tend to round up in mitosis. However, evidence from positively identified cells, mapped after staining their nuclei and chromosomes with Feulgen, methyl green-pyronin, orcein, or Giemsa reveals that cells in interphase can account for more than half of rounded cells on the coverslip and that up to 10% of division figures can occur in unremarkable flattened cells.

Nonadherent cells from the supernatant above the monolayer cultures and washed off by the water rinses and fixative were nearly all rounded and mostly in interphase. Cells of a cultured human lymphoid line (F-265) which grows in suspension were generally spherical with varied numbers of small microvilli, and a single ruffled region was present on most cells; mitotic cells of this line could not be readily distinguished by their surface features. The importance of visualizing cells fixed directly in suspension was emphasized by the striking morphological changes observed in these cells when they were allowed to settle for only one minute; the cells became flattened and asymetrical and the ruffles disappeared from the visible surfaces of the cells. By applying known numbers of fixed cells to the filters and counting the number of cells (per unit filtration area) retained on the filter for SEM, the overall yield of our method was shown to be on the order of 90%. We know of no other procedure for preparing cells fixed in suspension for SEM (without destructive air-drying) which promises such high yields.

Significance to Cancer Research:

SEM reveals fine surface structure with unparalled three-dimensionality, depth of field, and sampling capability. As this approach is adapted to biological problems through experience with diverse tissues, cellular form, surface details and interactions can be better interpreted in normal and malignant tissues, as well as in experimental systems which help define immune responses, differentiation, and other processes fundamental to oncogenesis and its control. The tissue culture studies directly elucidate new aspects of tumor cell biology and transformation.

Proposed Course of Project:

To further develop and refine techniques of specimen preparation, analysis, and interpretation for SEM will be the goal of continuing work with a wide variety of tissues, experimental approaches, and instrumentation.

Special emphasis will be accorded a search for novel cytochemical methods which are uniquely possible with this tool. Most of our attention will be shifted to tissue culture systems for these studies. Further collaboration in SEM, TEM and cytochemical studies of skin is under way with investigators in this branch and elsewhere.

Honors and Awards: None

Publications:

1) Wetzel, B.K., Cannon, G.B., Alexander, E.L., Erickson, B.W.,Jr., and Westbrook, E.W.: A Critical Approach to the Scanning Electron Microscopy of Cells in Suspension. SEM, IIT Research Institute, Chicago, Illonois, 1974.

Serial No. 3655
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Treatment of Basal Cell Carcinoma with Topical Vitamin A Acid

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1973 through June 30, 1974)

Total:	0.01
Professional:	0.01
Other:	0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical vitamin A acid in the treatment of patients with basal cell carcinoma.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone and in combinations) in the cure and palliation of cancer.

Agents:

0.5% to 3.0% vitamin A acid in water washable ointment base (unibase).

Material:

1) Basal Cell Carcinoma

2) Nevoid Basal Cell Carcinoma Syndrome

3) Xeroderma pigmentosum with cutaneous carcinoma

Methods Employed:

1) Patients must have pathologic confirmation of the clinical diagnosis.

2) They must have normal hematologic, hepatic, and renal function.

3) Lesions on eyelids and canthi are not included.

4) Patients are cautioned to avoid sun.

5) Ointments are applied twice daily to affected areas for 8 weeks.

6) Laboratory tests are done before, during, and 6 weeks after therapy.

7) Serial photographs are taken.

Major Findings:

1) In one patient, a 4X2 cm recurrent basal cell epithelioma resolved following treatment with topically applied 1% Vitamin A Acid. The lesion had been pretreated with superficial liquid Nitrogen cryotheropy 8 weeks previously. A hypertrophic scar developed at this site which involuted after 9 months. No recurrence has developed during the 3 year observation period.

2) In a second patient, 3% Vitamin A Acid was used to successfully treat a nodular basal cell carcinoma on the dorsum of the right hand.

3) In a third patient, a soletary large keratoacanthoma resolved clinically with the combination treatment of topical 1% Vitamin A Acid and 5% 5-fluorouracil. The lesion recurred, however, within a few months.

4) The back of a patient with xeroderma pigmentosum was divided in 4 quarters and treated with a) topical 5-FU alone, b) topical Vitamin A Acid alone, c) Both agents, or d) neither agent. Results indicated that the combination of agents produced the most inflammation and resulted in the most loss of pigmentation. The cancer prophylactic value of this regimen has not yet been determined as no tumors have yet developed in any of the 4 quadrants.

5) A patient with multiple actinic keratoses was treated with either a) topical 5-Fu alone, b) topical Vitamin A Acid alone, or c) a combination of both agents. Results of all three treatment programs were similar in producing partial responses.

6) A patient with the Basal Cell Nevus Syndrome is being treated with the combination of topical vitamin A acid and topical 5-FU. Most tumors have responded well, a few have disappeared completely.

Significance to Cancer Research:

1) This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior agent to 5-Fluorouracil in propylene glycol which is in widespread topical use.

2) We would also hope to contribute to the knowledge of the clinical pharmacology of this agent. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies.

Proposed Course of Project:

1) Continued observation of test subjects to determine recurrence rate.

2) Continued study of new patients as they become available.

Honors and Awards: None

Publications: 1. Peck, G.L.: Hypertrophic scar after cryotherapy and topical tretinoin. Arch Derm 108:819-822, 1973.

Serial No. NCI-
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Chemistry and Biosynthesis of Mammalian Epidermal Tonofilaments

Previous Serial Number: None

Principal Investigator: Peter M. Steinert, Ph.D.

Other Investigators: None

Man Years (December 10, 1973 through June 30, 1974)

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives:

Develop procedures for:
1) The isolation and purification of the tonofilaments of bovine epidermis.

2) To determine the ultrastructure of the tonofilaments by electron microscopy and other physicochemical procedures such as X-ray diffraction, optical rotatory dispersion etc.

3) To investigate the chemical composition of the tonofilaments. Such a study will entail: a) Characterization of subunit composition by gel electrophoretic procedures b) Separation of individual polypeptide chains by standard protein chemical methods c) Determination of the molecular weights, amino acid compositions, amino terminal residues and partial amino acid sequence of all or selected composite polypeptide chains by standard protein chemical methods.

4) To develop a cell-free protein synthesis system in which to study the synthesis and regulation of synthesis of the tonofilaments in normal and abnormal mammalian epidermis. This will involve: a) Selection of a suitable experimental system, which may or may not be bovine in origin b) Isolation of polyribosomes and development of cell-free protein synthesis systems c) Investigation of possible regulatory aspects of tonofilament biosynthesis in the cell-free system.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning.

Major Findings and Methods Employed:

1) A preliminary procedure for the isolation of tonofilaments has been established. This involves homogenization of approximately $1 mm^3$ pieces of bovine epidermis in a pH 3 buffer, followed by serial centrifugation steps to remove cellular debris. These procedures will be monitored by electron microscopy and suitably modified until satisfactorily homogeneous preparations of tonofilaments are achieved.

2) The polypeptide chains that comprise the tonofilaments may be isolated directly from the epidermal tissue without prior isolation of the tonofilaments. This is achieved by extraction of the tissue in a buffer of 8M urea containing 2-mercaptoethanol. The extracted material which represents 30-40% of the tissue consists of only 3 bands when examined by acrylamide gel electrophoresis, which correspond exactly to the bands obtained when tonofilaments are treated with the urea buffer.

3) A procedure for the separation of these three polypeptide chains has been established by chromatography on DEAE-cellulose. Further studies are now in progress to determine molecular weights and amino acid compositions, etc. These procedures are based on methods developed elsewhere during the characterization of hair proteins.

Significance to Cancer Research:

The epidermis offers a unique opportunity for the study of tumors not only because of the quantitative importance of this tissue but also because of its accessibility. One of the major problems in studying malignancies of the epidermis has been the lack of a suitable biochemical marker. The tonofilaments are the most prominent intracellular components of all epidermal cells and therefore a study of their chemistry, structure and biosynthesis in both normal and abnormal epidermis will be of profound importance in studying tumors in this tissue.

Proposed Course of Project:

Continuing emphasis will be placed on the objectives outlined above.

Publications:

1) Steinert, P.M., and Rogers, G.E.: Characterization of the proteins of guinea pig hair and hair-follicle tissue. Biochem. J. 135: 759, 1973.

ANNUAL REPORT OF THE METABOLISM BRANCH
SUMMARY OF SIGNIFICANT ACTIVITIES
NATIONAL CANCER INSTITUTE

July 1, 1973 through June 30, 1974

The clinical research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, a broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasms as well as in experimental animal systems to determine the nature of the immune processes that are of importance in the surveillance against neoplastic cells and to define the nature of defects in the immunological system that result in an increased incidence of neoplasia. Major efforts in this area are also directed toward developing techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy. The second major goal of the Branch is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Both patients with neoplastic disease as well as those with non-neoplastic disorders that facilitate the development of techniques for the study of cell membranes, homeostatic mechanisms and metabolic derrangements of these control mechanisms are being investigated.

Detection of Circulating Tumor Associated Antigens

Sensitive quantitative radioimmunoassays for oncofetal proteins such as alpha-fetoprotein (AFP) and the beta chain of human chorionic gonadotropin (hCG-β) have been established and evaluated as tests for the diagnosis and for the monitoring of treatment of different forms of cancer. An elevated serum AFP level (> 40 ng/ml) was demonstrated in a significant number of the 700 patients with cancer studied including 72% of the patients with hepatocellular carcinoma, 75% of the patients with teratocarcinoma or embryonal cell carcinoma of the testis, in 23% of patients with pancreatic carcinoma and in a lower percentage of patients with gastric, colonic or broncogenic carcinoma studied. In contrast, only 1 of 500 controls or patients with chronic non-hepatic diseases had a level over 40 ng/ml. In recent studies it was shown that the combined determination of several oncofetal markers can significantly increase the detection rate of cancer, for example, among 37 gastric cancer patients studied, carcinoembryonic antigen (CEA) was elevated in only 24%, and AFP elevated in only 32%. However, since only 1 of the patients with an elevated AFP also had an elevated CEA. The combined assays raised the diagnostic sensitivity to 57%. In addition, in the study of over 100 patients with embryonal carcinoma or teratocarcinoma of the testis, only 75% of the patients had an elevated AFP level and only 72% had an elevated hCG-β level, whereas 90% had an elevated level of at least one of these marker proteins. The serial determination of the serum level of both AFP and hCG-β markers were shown to be of special value in monitoring the effectiveness of chemotherapy of testicular teratocarcinoma and embryonal cell tumors. Thirteen of the 17 patients with this form of cancer studied extensively had pretreatment elevations of both markers, two had an elevation of hCG-β alone and one had an elevation of

AFP alone. There was a marked reduction in both markers following initiation of therapy in 12 of the 13 cases with elevations of both markers initially. In two cases both markers declined but remained above the upper limit of the normal range. In 9 cases both markers declined but only one of the markers (AFP 3, hCG-β 6) remained elevated and the remaining marker fell to normal levels. In addition, with the recurrence of the tumor one of the markers remained at a normal level whereas the other returned to a very high level (5 cases). This discordant behavior of these tumor markers suggests that their cells of origin are probably different and underscores the need to follow both markers in evaluating the adequacy of therapy. The importance of the use of multiple sensitive immunoassays for tumor markers is also supported by the observation that 1/3 of the treated patients in the present group had an elevation of one of the markers at a time when there was no clinical evidence of residual disease. In each of these cases with an elevation of a tumor marker there was a recurrence of the tumor in the subsequent 2 to 12 months of observation.

Studies of AFP levels in over 500 patients with benign liver diseases have resulted in the demonstration of elevated AFP levels in 29% of the patients with classical viral hepatitis, 52% of the patients with subacute hepatic necrosis, and in a lower percentage of patients with other liver diseases including Laennec's and biliary cirrhosis. An elevated AFP level did not correlate with other tests of liver function but appears to measure liver regeneration, a function not assessed by other tests.

Finally, in other studies performed in conjunction with K. R. McIntire, the tumor load of monkeys with hepatocellular carcinoma was defined by the simultaneous quantitative measurement of AFP levels in the serum, the determination of the metabolic parameters of AFP by in vivo turnover studies, and by the determination of the rate of biosynthesis of AFP by hepatocellular tumor cells using tissue culture biosynthesis measurements.

Immunophysiology

A significant effort of the Metabolism Branch over the past year has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific circulatory and cellular immune response to antigens. Antibodies are produced by cells originating in the bone marrow that mature by interaction with a central lymphoid organ (Bursa of Fabricius or its mammalian equivalent) to form B lymphocytes which in turn terminally differentiate into cells synthesizing immunoglobulins. B lymphocytes may be identified because they have immunoglobulins as well as receptors for the third component of compliment and for antigen-antibody complexes incorporated into their cell surface membranes. Immunodeficiency diseases in man associated with hypogammaglobulinemia may represent defects in the formation of B cells (e.g., Bruton's agammaglobulinemia) or defects in the terminal differentiation of immunoglobulin bearing B cells into immunoglobulin producing and secreting cells (e.g., common variable immunodeficiency, isolated IgA deficiency). We have, therefore, developed a technique for the study of the terminal differentiation of B lymphocytes so that we might define the nature of this critical process and define defects in this process in patients with immunodeficiency disorders. In this technique, peripheral blood lymphocytes from patients and controls are washed free of serum immunoglobulin and then incubated in culture media for a 7 day period in the presence or absence of mitogens. The IgG, IgA and IgM molecules produced by the cells and secreted into the medium are then quantitated using double antibody radioimmunoassays for each of these immunoglobulin classes. This technique is of the same value in the study of B cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus dependent lymphocyte (T cell) maturation. Thus, in vitro techniques are now available to evaluate both major arms of the specific immune response in man. In normal individuals B cell maturation and immunoglobulin synthesis was initiated following the addition of pokeweed mitogen or salmonella typhosa lypopolysaccharide (LPS) to the culture. This terminal differentiation of B cells could be completely abrogated by irradiation of the cells (2000 R) or by the addition of cytosine arabinoside, mitomycin C, actinomycin D or puromycin to the culture media. These studies as well as time course studies suggest that a critical mitosis is required before a major increase in lymphocyte immunoglobulin synthesis is possible following exposure of these cells to pokeweed mitogen or LPS. In other studies dibutyryl cyclic AMP or factors which increase intracellular cyclic AMP such as isoproterenol or prostaglandin E_1 prevented B cell maturation and immunoglobulin synthesis and release whereas cyclic GMP stimulated this maturational step. Another finding of major significance is that the addition of "T" cell mitogens such as phytohemagglutinin, staphylococcal filtrate, or concanavalin A to a culture of normal lymphocytes and the B cell stimulant pokeweed mitogen completely inhibited B cell maturation and immunoglobulin synthesis. These observations indicate that activated T cells or their products can act as nonspecific inhibitory regulators of B cell maturation and antibody synthesis. The use of this technique of assessing B cell maturation in the study of patients with immunodeficiency disease is discussed below.

Studies of several aspects of cell mediated immunity were continued in patients with a variety of neoplastic or immunodeficiency diseases and in basic animal models. In vitro cellular immune function has been assessed by four basically different techniques: 1. The proliferative response of lymphocytes following stimulation with mitogens, antigens, and allogenic cells; 2. The production of biologically active soluble mediators (lymphokines) secreted by stimulated lymphocytes; 3. The cytotoxic-killer activity of both immune and non-immune lymphoid cells; and, 4. The in vitro responsiveness of mononuclear leukocytes (monocytes and macrophages) to various lymphokines and other biological mediators such as activated serum complement components.

An important in vitro correlate of cellular immune function is the ability of lymphocytes to kill various target tissues and cells, a role thought to be particularly important for host resistance to tumor cells bearing new antigens on their surface. Studies of the nature of the cells involved in these reactions and their mechanism of action were carried out in several basic animal model systems. Certain classes of antibody have the property that they induce nonimmune lymphocytes to attack and kill target cells coated with these antibodies. These antibodies have been termed lymphocyte dependent antibodies (LDA) because their biologic activity depends upon lymphocyte action just as complement dependent antibodies require complement for their biologic activity. A question of central importance in the understanding of the mechanism of this form of lymphoid cell killer activity is the determination of the type of cell doing the killing. Studies of patients with a variety of T cell defects failed to disclose a consistent pattern of reactivity. However, patients with intestinal lymphangiectasia were found to have significantly enhanced LDA killer cell activity in their peripheral blood and depressed killer activity by the lymphocytes isolated from their chylous effusions. This differential killer activity of these two lymphocyte pools was the exact converse of the in vitro proliferative activity which we had previously observed in these same lymphocyte pools suggesting that LDA killer cells were predominantly from the non-recirculating lymphocyte population (B-lymphocytes) whereas the lymphocytes responding with blastogenic transformation were from the long lived recirculating population of T cells. Further studies of LDA killer activity by lymphocytes from patients with hypogammaglobulinemia usually showed a marked depression of this function while the lymphocyte proliferative responses from these patients were more nearly normal. However, certain hypogammaglobulinemic patients did have normal LDA killer activity. To define the cause of the variability of response of lymphocytes from hypogammaglobulinemic patients, the cell surface receptor markers on B lymphocytes from such patients were compared with the degree of LDA killer activity observed. It was found that those hypogammaglobulinemic patients who lacked both immunoglobulin receptors and receptors for IgG-Fc on their lymphocytes had the most profound depression in LDA killer activity, whereas those hypogammaglobulinemic patients whose lymphocytes had a normal compliment of IgG-Fc receptors killed normally. Thus, the LDA killer cell activity in man appears to reside in the non-recirculating B lymphocyte population and appears to be dependent upon a normal IgG-Fc receptor on these cells. It was also observed that antigen-antibody complexes or aggregated IgG (with activated Fc components) could effectively block the killer activity of these cells, presumably by competitively blocking the Fc receptor site on these killer cells.

Another in vitro test for competence of cell mediated killing has been developed following the observation that normal nonimmune lymphocytes will attack and kill certain target tissues or cells if incubated together in the presence of phytomitogens like PHA. The killing observed in this system occurs very rapidly and therefore is apparently measuring the cytotoxic potential of a pre-existing killer cell rather than causing the generation of new killers. It was also found that killing observed in this system was dependent on a direct link being formed between the killer cell and the target via the mitogen. Thus, although concanavalin A was capable of inducing lymphocytes to kill burro red cells, it was ineffective in inducing lysis of human red cells. Conversly, pokeweed mitogen induced lymphocytes to kill human but not burro red cells. By appropriate mixture and absorption studies it was shown that both the killer cell and the target cell must have receptors on their surfaces for the mitogen to allow a cross-linking between the two, and that the killing observed could not be due to the release of a toxic lymphocyte mediator such as lymphotoxin. Since PHA and ConA are felt to be exclusively T cell mitogens, it was believed that the killer cell detected in these studies must, therefore, be a T lymphocyte. In studies utilizing lymphocytes from agammaglobulinemic chickens, it was shown that those cells were just as effective as cells from normals, at least indicating that "B" lymphocytes are not required for this type of target cell killing. It was also found that cell suspensions depleted of all phagocytic cells were also effective killers so that monocytes, macrophages, and PWM's are also not required to obtain mitogen activated target cell killing. However, in recent studies it was also shown that bone marrow lymphocytes from athymic nude mice also can effectively kill these red cell targets in the presence of phytomitogens. Thus, cell populations depleted specifically of all three elements - T cells, B cells, and phagocytes - all retain mitogen induced killer cell activity suggesting that this form of cytotoxicity may reflect a much more undifferentiated function than previously supposed. In this regard, it is particularly interesting that a number of patients with immunodeficiency disease or malignancy do have severely depressed ability to kill targets via this mechanism.

During the past year the Immunophysiology Section of the Metabolism Branch continued studies on normal immunologic processes of the gastrointestinal tract as well as studies on abnormal immunologic processes encountered in various gastrointestinal diseases. In the area of normal gastrointestinal immunology the functional capacity of organized gut-associated lymphocytes (Peyer's patch lymphocytes) was investigated. It was shown that in common with other lymphoid tissue, guinea pig Peyer's patches contained cells which bear a receptor for sheep erythrocytes. These cells undergo proliferation when exposed to a variety of nonspecific mitogens, including Concavalin A, phytohemagglutinin and pokeweed mitogen. In addition, these cells can be induced by these mitogens to become cytotoxic effector cells with activity against various erythrocyte and epithelial cell targets. These studies indicate that Peyer's patches contain functional T cells with cytotoxic capability. In other studies, it was shown that lymphocytes from Peyer's patches bear receptors for aggregated guinea pig gammaglobulin. However, unlike other cell populations which also contain aggregate-marker cells, Peyer's patches cells cannot be enlisted by specific antibody to become cytotoxic effector cells. These results may be due to the fact that B cells within Peyer's patches are functionally deficient.

Immunodeficiency and Malignancy

Studies of patients with immunodeficiency disease and a high incidence of malignancy including patients with the Wiskott-Aldrich syndrome, hypogammaglobulinemia, ataxia telangiectasia and intestinal lymphangiectasia are continuing with the ultimate goal of correction of the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease.

The Wiskott-Aldrich syndrome is an x-linked immunodeficiency disorder with a very high incidence of infections with all classes of microorganisms and, in addition, the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that the effector arms of their immune system is largely intact. The patients have normal numbers of circulating T and B lymphocytes as detected by immunofluorescence or immunocytotoxicity assay with specific antisera. They produce immunoglobulins at normal or elevated rates. Their lymphocytes have normal proliferative responses *in vitro* to non specific mitogens and they produce lymphokines such as macrophage migration inhibitory factor (MIF), lymphotoxin and chemotactic factor for monocytes normally. In addition, the killer function of their B lymphocytes is normal as detected by *in vitro* antibody directed lymphocyte mediated cytotoxicity, and their T lymphocyte killer cells are normal as tested in PHA activated killing of ^{51}Cr-labeled target cells *in vitro*. In spite of these normal *in vitro* tests these patients lack adequate specific humoral and cellular immune responses both *in vitro* and *in vivo* which supports our view that the immunodeficiency of this disorder is the result of a defect in afferent limb of immunity, perhaps a defect in antigen recognition or processing or a defect in control of immune responses. Defective control of immunoresponsiveness is also suggested by the observation that many of these patients have developed transient monoclonal gammopathy. In addition, the unstimulated lymphocytes from these patients studied by *in vitro* culture techniques were found to spontaneously produce as much of a chemotactic factor for monocytes as do lymphocytes from normal individuals only after antigen or mitogen stimulation. This high level of unstimulated chemotactic factor production apparently contributes to the anergy in these patients by producing high circulating levels of chemotactic factor which tends to abolish any chemotactic gradient produced by a specific immune response and also be deactivating the chemotactic responsiveness of the monocytes from these patients.

A most striking feature of the Wiskott-Aldrich syndrome is the very high incidence of lymphoreticular malignancy found in these patients. Six of the 13 patients studied at the NIH since 1966 have developed malignancy. In a very provocative study in collaboration with Drs. K. K. Takomoto and A. S. Rabson, we have isolated a papovavirus from a reticulum cell sarcoma involving the brain of one of our patients. Serum from this patient was studied for antibody to the virus and about 14 months prior to the clinical appearance of the tumor the patient developed a striking antibody response to this virus. Following the isolation of this papovavirus from the child's brain tumor, the virus was also isolated from his urine on each of 3 attempts. At post mortem examination, 5 months after the initial brain tumor virus isolation, the patient was found to have massive tumorous invasion of the kidneys, perhaps explaining the presence of virus in the urine. The isolation of a virus belonging to the papova group, which are well-known for their oncogenic properties, from a human tumor raises intreging questions in regard to the possible role of these viruses in human cancer. Although this single observation certainly does not prove an etiologic relationship between this virus and cancer, it does provide a strong stimulus to further investigation of its association with malignancy, particularly that occurring in immunodeficient individuals.

The technique for the study of the terminal differentiation of B cells into immunoglobulin synthesizing cells discussed above was used in studies directed toward defining the nature of the immunological defect in patients with common variable immunodeficiencies (acquired hypogammaglobulinemia), patients with ataxia telangiectasia and patients with isolated IgA deficiency. Patients with common variable hypogammaglobulinemia are unable to produce antibodies to antigenic challenge and have markedly depressed rates of antibody production. A high percentage of these patients, however, were shown to have normal numbers of B cells, that is, lymphocytes having cell surface receptors for complement for antigen-antibody complexes as well as normal quantitites of cell surface immunoglobulin that function as a membrane bound receptor for antigen. These patients, thus, have a disorder in terminal differentiation of these B cells into cells that can synthesize and secrete immunoglobulins. When studied using the in vitro culture technique the lymphocytes of such patients were not able to make IgG, IgA or IgM following stimulation with pokeweed mitogen. Of even greater interest, when lymphocytes from such hypogammaglobulinemic patients were incubated with normal lymphocytes and pokeweed mitogen the synthesis of immunoglobulin by the normal cells was prevented suggesting that patients with acquired hypogammaglobulinemia have a cell (presumably a T cell) that actively suppresses B cell maturation and antibody production. Patients with isolated IgA deficiency or with ataxia telangiectasia and IgA deficiency were shown to have normal numbers of B cells that bear IgA molecules on their cell membrane. Thus, these patients again appear to have a defect in the terminal differentiation of IgA bearing B cells into IgA secreting plasma cells. In the in vitro culture system with pokeweed mitogen the majority of these patients produced cells in their cytoplasm but no secretion of IgA into the media was observed. The studies suggest that limited IgA synthesis is possible in response to pokeweed mitogen but release into the media is inhibited with these patients. In general, such patients did not have circulating or cellular inhibitors of B cell maturation nor could they be made to synthesize and secrete IgA into the media. Two interesting exceptions to this generalization were demonstrated. In one patient who had a very high titer of antibodies to IgA there was a circulating inhibitor to the maturation and secretion of IgA by normal cells when they were incubated in the presence of serum from this patient. The second patient had a severe defect in thymic function as well as a defect in IgA production. The cells of this patient did not make significant quantities of IgA when cultured with pokeweed mitogen. However, when T cells from another individual that did not themselves synthesize IgA were added to the culture system, the cells of the patient produced large quantities of IgA indicating that there may be a requirement for cooperation of T cells and B cells in terminal differentiation and secretion of IgA by appropriate B cells.

Studies of the immunologic defects in the syndrome intestinal lymphangiectasia have continued. Intestinal lymphangiectasia is a disease characterized by a disorder of lymphatic channel development leading to dilation of the lymphatic channels of the small bowel, the hallmark morphologic lesion of this disease. This defect is associated with bulk loss of lymphatic fluid into the gastrointestinal tract. Patients with intestinal lymphangiectasia have demonstrated profoundly impaired peripheral blood lymphocyte proliferative responses in vitro to all classes of mitogenic stimuli. We have postulated that a qualitative defect in the lymphocyte pool of these patients results from the selective depletion of the long lived recirculating T lymphocytes by gastrointestinal lymph loss should result in reversal of the immunodeficiency in these patients. We have recently had the opportunity to test this hypothesis directly in a patient whose intestinal lymphangiectasia was secondary to constrictive pericarditis. This patient had profound hypoproteinemia and lymphocytopenia with marked clinical cellular immunodeficiency as determined by failure to reject a skin allograft and inability to mount any delayed type hypersensitivity reaction. Following successful pericardectomy the patient's gastrointestinal protein loss ceased, his peripheral lymphocyte count returned to normal, he developed positive delayed hypersensitivity reactions to many common environmental antigens, the skin allograft was rejected, and the *in vitro* reactivity of his peripheral lymphocytes returned to normal. Thus, our experience with this patient directly demonstrates that the entire immunodeficiency syndrome associated with intestinal lymphangiectasia can be accounted for by the loss of immune effectors into the gastrointestinal tract.

Patients with chronic lymphocytic leukemia (CLL) also have profound defects in *in vitro* T cell and B cell lymphocyte function and frequently have hypogammaglobulinemia and clinical anergy as well. The predominant circulating lymphocyte in these patients is a B cell bearing homogenous 8S IgM on their surface. Peripheral blood lymphocytes from such patients produce low quantities of IgM and exceedingly low quantities of IgG and IgA in culture with pokeweed mitogen. In addition lymphocytes from CLL patients generally fail to proliferate in response to T cell mitogens, antigens or allogeneic cells. They also are unable to produce biologically active lymphokines and their lymphocytes are ineffective as killer cells in a variety of *in vitro* cytotoxicity assays. To determine if the profound *in vitro* deficiency of cells from these patients reflected a general lack of immunocompetent lymphocytes or alternatively, dilution of normal cells by a great excess of leukemic nonfunctional cells, normal T lymphocytes were isolated from the blood of these patients by their ability to form spontaneous rosettes with sheep red blood cells. Isolated T cells from these patients were found to respond almost normally in each of the T cell assays which showed profound deficiency when their unseparated lymphocytes were tested. Thus, dilution of normally reactive lymphocytes with nonfunctional leukemic B cells appears to account for the general failure of CLL cells to proliferate, produce lymphokines, and to behave as cytotoxic effector cells *in vitro*.

Patients with Hodgkin's and non-Hodgkin's lymphoma have a variable degree of *in vivo* and *in vitro* immunodeficiency. Patients with the most advanced disease were found to have diminished numbers of circulating T lymphocytes and decreased *in vitro* proliferative responses, particularly when stimulated with specific antigens *in vitro*. Killer lymphocyte function in many of these patients was also defective while their ability to produce a lymphokine chemotactic for monocytes was generally intact. The responsiveness of monocytes from these patients to chemotactic factors was also frequently abnormal. During the course of therapy, these patients having a beneficial response showed remarkable recovery of *in vitro* cellular immune function, while those patients who were treatment failures also failed to develop improved cellular immune function *in vitro*. It, thus, appears that study of *in vitro* lymphocyte function may provide a useful prognostic index for the immediate prospects of remission in these lymphoid malignancies, although only longer periods of follow-up will indicate the long term prognostic implications of these observations.

In the area of abnormal gastrointestinal immunology, studies on the pathogenesis of gluten-sensitive enteropathy were continued. Gluten sensitive enteropathy is a disease of the gastrointestinal tract associated with malabsorption, villous atrophy, and an increased incidence of neoplasia. We have shown that patients with this disease produce antibody with gluten specificity at mucosal sites following exposure to dietary gluten. In the current series of experiments the relationship of this finding to the pathogenesis of the disease was investigated. First, we noted that in organ cultures of jejunal tissue, brush border alkaline phosphatase increases, probably as a result of cell maturation. In tissues of patients with active gluten sensitive enteropathy such increases were again seen but could be prevented by the addition of gluten-peptides to the culture medium. This prevention of cell maturation in culture by gluten-peptides constitutes an *in vitro* model of gluten sensitive enteropathy.

Whereas gluten-peptides affected cell maturation in tissue from patients with active disease it had no effects on cell maturation in tissue from patients with inactive disease, i.e., patients on a gluten-free diet. This suggests that gluten is not directly toxic to the GI mucosa of GSE patients but requires the activation of an endogenous mechanism. To substantiate this possibility, tissue from patients with active disease was cultured together with tissue from patients with inactive disease in the presence and absence of gluten-peptides. It was observed that although gluten-peptides had no effect on inactive tissue culture alone it prevented maturation of inactive tissue cultured in the presence of active tissue. Control mixture experiments using normal tissue in place of active tissue did not have a similar effect. These experiments show that tissue from patients with active GSE elaborates a soluble factor which, in concert with gluten-peptides, prevents tissue maturation in GSE tissue. It is reasonable to assume that this soluble factor is antibody with gluten specificity.

In other studies the relationship between histocompatability type and gluten-sensitivity disease was further explored. It was found earlier that in dermatitis herpetiformis (DH), a skin disease associated with gluten sensitive enteropathy in many instances, HL-A8 occurred at a generally increased frequency. In the present studies, it was shown that in two separate populations HL-A8 was especially frequent (80-90% incidence) in those patients with DH who have gastrointestinal disease and infrequent in those patients with DH who do not have gluten-sensitive disease (20%). The implication of this finding is that HL-A8 acts in cooperation with other genes to produce gluten sensitive disease.

The relationship between HL-A8 and gluten sensitive disease was also explored in organ culture experiments. It was observed that gluten-peptides prevents tissue maturation in biopsy tissue of active patients in organ culture only if such tissue was obtained from patients having HL-A8. If tissue from active patient not bearing HL-A8 was studied, gluten-peptides had no effect on tissue maturation in culture. This finding is probably related to subtle differences in disease mechanisms of HL-A8 positive and negative individuals with GSE.

Porphyrin Metabolism

A method for the measurement of uroporphyrinogen I synthetase in red cells has been developed. The method has been shown to separate heme from the porphyrins and thereby eliminate errors caused by quenching from heme. Because of the high degree of separation of heme from porphyrins the method is adaptable to various volumes and dilutions of enzyme and can be used in different tissues. The optimal conditions for measurement of the enzyme have been worked out in detail and the method is highly reproducible. A study of the level of this enzyme in the red cells of 9 patients with acute intermittent porphyria compared with 28 normals showed the mean value in the patients with acute porphyria to be close to 1/2 the mean value in normals. The range of values in the patients with acute porphyria did not overlap the range of normal values. In 10 children with a known "porphyric" parent the enzyme level was normal in 5, clearly in the "porphyric" range in 4 and questionable in one. This is the expected incidence of porphyria. The children with a "positive" enzyme test had urines which were "negative for porphyria." These findings clearly indicate that the enzyme test will be of great diagnostic value for acute intermittent porphyria and that it can detect the disease in asymptomatic individuals when chemical analysis of the urine fails to establish the diagnosis. Furthermore, the test can distinguish acute intermittent from other varieties of porphyria, since the levels of red cell enzyme in these types of porphyria was shown to be well within the normal range.

The presence of a substance in tumors (9 different rat tumors) which blocks the induction of hepatic ALA synthetase was strongly suggested by previous studies in this laboratory. A system for the detection of this substance in tumor fractions has been devised using the induction of ALA synthetase in chick embryo liver. Preliminary work on the characterization and isolation of this substance is in progress.

A mathematical model for the control of enzyme synthesis has been developed, utilizing a chemical closed negative feedback loop. The system of non-linear differential equations which describes the behavior of the components of the system can not be solved analytically. A collaborative study with Dr. Mones Berman's group was begun using the SAAM program for computer solution of the variables under various conditions. It was shown that this type of system exhibits a high degree of stability, but that oscillatory changes in enzyme levels can be produced by certain perturbations of the system.

Proline Transport, Biosynthesis and Metabolism

In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase.

We have developed specific, sensitive radioisotopic assays for the four aforementioned enzymes, using labelled substrates and quantitating the products formed by ion exchange chromatography. With these assays we have measured the activities of the proline metabolic enzymes in circulating leukocytes, in cultured cells undergoing a variety of perturbations. Our findings can be grouped into three major areas:

Phenotypic Alterations in PC Reductase. Regulation of amino acid biosynthetic-enzymes in mammalian cells remains poorly understood. Our studies show that PC reductase, the enzyme catalyzing the committed step in proline biosynthesis is regulated by a novel mechanisms. We previously reported PC reductase was sensitive to proline feedback inhibition in rapidly growing cultured cells but was insensitive to proline inhibition in tissues. We have now shown that in resting cells undergoing growth activation, PC reductase is converted from the proline insensitive to the proline sensitive form. Resting lymphocytes have a PC reductase which is inhibited only 20-30% by 10^{-3}M proline. After 72 hours of incubation with phytohemagglutinin, a well known activator of lymphocytes, lymphocytes show a 15-fold increase in PC reductase. Of greater interest, the enzyme becomes 90% inhibited by 10^{-3}M proline. Over the 72-hour period the increase in PC reductase parallels the appearance of proline sensitive enzyme. Cycloheximide or actinomycin D will block both the increase in activity and the appearance of proline sensitivity. These findings suggest that activation of resting cells includes the expression of new genes producing a phenotypically altered PC reductase. In activated cells, the increased activity of the enzyme allows for maximal proline synthesis while the sensitivity of the enzyme to proline inhibition spares precursors vital for other cellular functions.

Type II - Hyperprolinemia - PC Dehydrogenase Defect. Patients with Type II hyperprolinemia are characterized by a high plasma proline concentration (10 X normal) and overflow iminoglycinuria. In addition, they excrete in the urine large amounts of O-aminobenzaldehyde reactive material, presumably PC. Fibroblasts were obtained from two unrelated patients with Type II hyperprolinemia. Both had undetectable PC dehydrogenase activity as compared to normal activities found in fibroblasts of 4 normal subjects. By contrast, the activities of ornithine-δ-transaminase and PC reductase activities were the same in Type II hyperprolinemia fibroblasts as in normals. This metabolic defect can also be diagnosed in circulating leucocytes. We obtained leucocytes from one patient with Type II hyperprolinemia, her parents and her three siblings. As compared to leucocytes from 10 normal subjects (PC dehydrogenase activity, 40 ± 2 nMoles/hr/mg protein) the propositus had undetectable levels; mother, 10 nMoles/hr/mg protein; father, 25 nMoles/hr/mg protein and the three siblings, 23, 23, and 21 nMoles/hr/mg protein.

Viral Transformation. Proline oxidase, a hepatic mitochondrial enzyme converting proline to Δ^1-pyrroline-5-carboxylate, is one of the enzymes involved in gluconeogenesis. In cultured mammalian fibroblasts proline oxidase activity is undetectable throughout the growth cycle. In SV40 transformed fibroblasts, however, significant enzyme activity can be measured in both whole homogenates and isolated mitochondria. The activity of ornithine-δ-transaminase, another mitochondrial enzyme, is identical in normal and SV40 transformed fibroblasts.

We are studying this enzyme in several other transformed fibroblast lines to ascertain if the presence of proline oxidase is a marker enzyme for transformation.

Mechanism of Action of Growth Hormone

An understanding of the biochemical events accompanying normal growth (as mediated by growth hormone, for example) may be a prerequisite for understanding malignant growth. There is evidence that the anabolic action of growth hormone is mediated by a second protein factor designated somatomedin. Somatomedin may also be one of the serum factors required for the growth of cells in culture. Work in the laboratory over the past year has been directed toward purification of somatomedin from the conditioned medium of a Buffalo rat liver cell line that grows without serum and, in the development of a suitable tissue culture system in which to study the biochemical events accompanying growth stimulated by somatomedin. DNA synthesis in the chick embryo fibroblast as measured by incorporation of radioactive thymidine has been shown to be responsive to growth hormone dependent serum factor(s). The incorporation of thymidine stimulated by hypophysectomized rat serum is only 20% of the level resulting from addition of normal serum. Serum from growth hormone treated hypophysectomized rats restores thymidine incorporation to at least 60% of the normal level. This growth hormone dependent factor which restores thymidine incorporation is nondialysable and heat stable. Mixing experiments have shown that the low level of thymidine incorporation seen with hypophysectomized rat serum is not due to the presence of an inhibitor. Growth hormone does not stimulate thymidine incorporation when added directly to chick embryo fibroblasts *in vitro*. Thus, chick embryo fibroblasts may be a suitable *in vitro* system in which to study the anabolic action of somatomedin.

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Individual Project Report
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Project Title: Studies of Physiology and Pathophysiology of Plasma Protein Metabolism

Previous Serial Number: SAME

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Warren Strober, M.D., R. Michael Blaese, M.D., K. Robert McIntire, M.D. and Samuel Broder, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total: 4
Professional: 2
Others: 2

Project Description:

Objectives: The objectives of the study were: to determine the normal rates of synthesis, transport and catabolism of the plasma proteins, especially the immunoglobulins, to determine the physiological factors controlling these metabolic parameters and to determine the pathophysiology of disorders of plasma protein metabolism in patients with neoplastic disease and in patients with hereditary disorders of immunoglobulin metabolism In addition studies were directed toward determining the sequential steps involved in the differentiation of lymphocyte B-cells to immunoglobulin synthesizing and secreting cells. Finally, studies were directed toward developing techniques for the detection of circulating tumor-related antigens.

Methods Employed: The rates of synthesis, catabolism and distribution of the serum proteins were studied using radioiodinated labeled purified proteins. Double antibody radioimmunoassays were established to detect α-fetoprotein, human chorionic gonadotropin and the immunoglobulins IgG, IgA, IgM, IgD and IgE. As indicated below, a new technique has been developed for the study of the terminal differentiation of B lymphocytes to immunoglobulin secreting cells.

Major Findings: A significant effort over the past year has been directed toward defining the nature of the events of cellular differentiation, cellular interactions and cellular biosynthesis involved in the specific

Patients with common variable hypogammaglobulinemia are unable to produce antibodies to antigenic challenge and have markedly depressed rates of antibody production. A high percentage of these patients, however, were shown to have normal numbers of B-cell, that is, lymphocytes having cell surface receptors for complement for antigen-antibody complexes as well as normal quantities of cell surface immunoglobulin that function as a membrane bound receptor for antigen. These patients, thus, have a disorder in terminal differentiation of these B-cells into cells that can synthesize and secrete immunoglobulins. When studied using the in vitro culture technique the lymphocytes of such patients were not able to make IgG, IgA or IgM following stimulation with pokeweed mitogen. Of even greater interest, when lymphocytes from such hypogammaglobulinemic patients were incubated with normal lymphocytes and pokeweed mitogen the synthesis of immunoglobulin by the normal cells was prevented suggesting that patients with acquired hypogammaglobulinemia have a cell (presumably a T-cell) that actively suppresses B-cell maturation and antibody production. Patients with isolated IgA deficiency or with ataxia telangiectasia and IgA deficiency were shown to have normal numbers of B-cells that bear IgA molecules on their cell membrane. Thus, these patients again appear to have a defect in the terminal differentiation of IgA bearing B-cells into IgA secreting plasma cells. In the in vitro culture system with pokeweed mitogen the majority of these patients produced cells with IgA in their cytoplasm but no secretion of IgA into the media was observed. The studies suggest that limited IgA synthesis is possible in response to pokeweed mitogen but release into media is inhibited with these patients. In general, such patients did not have circulating or cellular inhibitors of B-cell maturation nor could they be made to synthesize and secrete IgA into the media. Two interesting exceptions to this generalization were demonstrated. In one patient who had a very high titer of antibodies to IgA there was a circulating inhibitor to the maturation and secretion of IgA by normal cells when they were incubated in the presence of serum from this patient. The second patient had a severe defect in thymic function as well as a defect in IgA production. The cells of this patient did not make significant quantities of IgA when cultured with pokeweed mitogen. However, when T-cells from another individual that did not themselves synthesize IgA were added to the culture system, the cells of the patient produced large quantities of IgA indicating that there may be a requirement for cooperation of T-cells and B-cells in terminal differentiation and secretion of IgA by appropriate B-cells. The immunoglobulin synthesis of peripheral blood B lymphocytes was also performed in patients with the Sezary syndrome, chronic lymphocytic leukemia and multiple myeloma. Patients with the Sezary syndrome had essentially no immunoglobulin synthesis by their peripheral blood lymphocytes. The lymphocytes of patients with chronic lymphocytic leukemia synthesized low quantities of IgM in the absence of mitogens and virtually no IgG and IgA. There was no stimulation of immunoglobulin synthesis following addition of pokeweed mitogen. Patients with multiple myeloma had a low level of synthesis of polyclonal immunoglobulin and approximately normal levels of the immunoglobulin and approximately normal levels of synthesis of the immunoglobulin class comparable to their paraprotein. A small percentage

(20-30%) of this represented synthesis of the specific paraprotein. Patients with macroglobulinemia of Waldenström showed a variable pattern. A majority of the patients showed reduced polyclonal IgG and IgA synthesis and exceedingly high levels of IgM synthesis by their peripheral blood lymphocytes.

Another area of major effort was directed toward developing techniques for the detection of circulating tumors associated antigens that may be of value in the diagnosis of malignancy and monitoring therapy of cancer. Sensitive quantitative radioimmunoassays for oncofetal proteins such as α-fetoprotein (AFP) and the β-chain of human chorionic gonadotropin (hCG-β) have been established and evaluated as tests for the diagnosis and for the monitoring of treatment of different forms of cancer. An elevated serum AFP level (> 40 ng/ml) was demonstrated in a significant number of the 700 patients with cancer studied including 72% of the patients with hepatocellular carcinoma, 75% of the patients with teratocarcinoma or embryonal cell carcinoma of the testis, in 23% of patients with pancreatic carcinoma and in a lower percentage of patients with gastric, colonic or broncogenic carcinoma studied. In contrast, only 1 of 500 controls or patients with chronic nonhepatic diseases had a level over 40 ng/ml. In recent studies it was shown that the combined determination of several oncofetal markers can significantly increase the detection rate of cancer, for example, among 37 gastric cancer patients studied, carcinoembryonic antigen (CEA) was elevated in only 24%, and AFP elevated in only 32%. However, since only 1 of the patients with an elevated AFP also had an elevated CEA. The combined assays raised the diagnostic sensitivity to 54%. In addition, in the study of over 100 patients with embryonal carcinoma or teratocarcinoma of the testis, only 75% of the patients had an elevated AFP level and only 72% had an elevated hCG-β level, whereas 90% had an elevated level of a least one of these marker proteins. The serial determination of the serum level of both AFP and hCG-β markers were shown to be of special value in monitoring the effectiveness of chemotherapy of testicular teratocarcinoma and embryonal cell tumors. Thirteen of the 17 patients with this form of cancer studied extensively had pretreatment elevation of both markers, two had an elevation of hCG-β alone and one had an elevation of AFP alone. There was a marked reduction in both markers following initiation of therapy in 12 of the 13 cases with elevations of both markers initially. In two cases both markers declined but remained above the upper limit of the normal range. In 9 cases both markers declined but only one of the markers (AFP 3, hCG-β 6) remained elevated and the remaining marker fell to normal levels. In addition, with the recurrence of the tumor one of the markers remained at a noraml level whereas the other returned to a very high level (5 cases). This discordant behavior of these tumor markers suggests that their cells of origin are probably different and underscores the need to follow both markers in evaluating the adequacy of therapy. The importance of the use of multiple sensitive immunoassays for tumor markers is also supported by the observation that 1/3 of the treated patients in the present group had an elevation of one of the markers at a time when there was no clinical evidence of residual disease. In each of these cases with an elevation of a tumor marker there was a circulatory and cellular immune response to antigens. Antibodies are produced by cells originating in the bone marrow that mature by interaction with a central lymphoid organ (Bursa of Fabricius or its mammalian equivalent) to form B lymphocytes which in turn terminally differentiate into cells synthesizing immunoglobulins. B lymphocytes may be identified because they have immunoglobulins as well as receptors for the third component of complement and for antigen-antibody complexes incorporated into their cell surface membranes. Immunodeficiency diseases in man associated with hypogammaglobulinemia may represent defects in the formation of B-cells (e.g., Bruton's agammaglobulinemia) or defects in the terminal differentiation of immunoglobulin bearing B-cells into immunoglobulin producing and secreting cells (e.g., common variable immunodeficiency, isolated IgA deficiency). We have, therefore, developed a technique for the study of the terminal differentiation of B lymphocytes so that we may define the nature of this critical process and define defects in this process in patients with immunodeficiency disorders. In this technique, peripheral blood lymphocytes from patients and controls are washed free of serum immunoglobulin and then incubated in culture media for a 7 day period in the presence or absence of mitogens. The IgG, IgA and IgM molecules produced by the cells and secreted into the medium are then quantitated using double antibody radioimmunoassays for each of these immunoglobulin classes. This technique is of the same value in the study of B-cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus-dependent lymphocyte (T-cell) maturation. Thus, in vitro techniques are now available to evaluate both major arms of the specific immune response in man. In normal individuals B-cell maturation and immunoglobulin synthesis was initiated following the addition of pokeweed mitogen or salmonella typhosa lypopolysaccharide (LPS) to the culture. This terminal differentiation of B-cells could be completely abrogated by irradiation of the cells (2000 R) or by the addition of cytosine arabinoside, mitomycin C, actinomycin D or puromycin to the culture media. These studies as well as time course studies suggest that a critical mitosis is required before a major increase in lymphocyte immunoglobulin synthesis is possible following exposure of these cells to pokeweed mitogen or LPS. In other studies dibutyryl cyclic AMP or factors which increase intracellular cyclic AMP such as isoproterenol or prostaglandin E_1 prevented B-cell maturation and immunoglobulin synthesis and release whereas cyclic GMP stimulated this maturational step. Another finding of major significance is that the addition of "T" cell mitogens such as phytohemagglutinin, staphylococcal filtrate, or concanavalin A to a culture of normal lymphocytes and the B-cell stimulant pokeweed mitogen completely inhibited B-cell maturation and immunoglobulin synthesis. These observations indicate that activated T-cells or their products can act as non-specific inhibitory regulators of B-cell maturation and antibody synthesis. The technique for the study of the terminal differentiation of B-cells into immunoglobulin synthesizing cells was used in studies directed toward defining the nature of the immunological defect in patients with common variable immunodeficiency (acquired hypogammaglobulinemia), patients with ataxia-telangiectasia and patients with isolated IgA deficiency.

recurrence of the tumor in the subsequent 2 to 12 months of observation.

Studies of AFP levels in over 500 patients with benign liver diseases have resulted in the demonstration of elevated AFP levels in 29% of the patients with classical viral hepatitis, 52% of the patients with subacute hepatic necrosis, and in a lower percentage of patients with other liver diseases including Laennec's and biliary cirrhosis. An elevated AFP level did not correlate with other tests of liver function but appears to measure liver regeneration, a function not assessed by other tests.

Finally, in other studies performed in conjunction with K.R. McIntire, the tumor load of monkeys with hepatocellular carcinoma was defined by the simultaneous quantitative measurement of AFP levels in the serum, the determination of the metabolic parameters of AFP by in vivo turnover studies, and by the determination of the rate of biosynthesis of AFP by hepatocellular tumor cells using tissue culture biosynthesis measurements.

Significance to Biomedical Research: The technique developed for the study of the terminal differentiation of B-cells is of the same value in the study of B-cell maturation as is the lymphocyte blastogenic response to PHA in the study of thymus-dependent lymphocyte (T-cell) maturation. Thus, in vitro techniques are now available to evaluate both arms of specific immune response in man. One can now study the physiological factors regulating this response, one can define defects in the process in patients with immunodeficiency disorders or neoplasia of the B-cell or T-cell lymphocyte system. In addition, such studies can be used to determine the effect of chemotherapeutic or other agents of the terminal differentiation of B-cells in parallel with studies of the effects of these agents on T-cells so that one can define agents which preferentially affect T-cell immunity and those which preferentially effect B-cell immunity. Detection of circulating tumor-related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of the neoplastic disease and determining the efficacy of therapy of these disorders.

Proposed Course: Studies directed toward understanding the pathways and controlling mechanisms of the synthesis, transport and catabolism of serum proteins will be continued. Special emphasis will be placed on defining the physiological and pathophysiological controlling the sequential development of B-cells into immunoglobulin synthesizing cells. Special emphasis will also be placed on the development of new techniques for the detection of circulating tumor-related antigens. These latter studies will include the evaluation of the synthetic rates of catabolism of such tumor-related antigens.

Honors and Awards

None

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Serial No. NCI-4003(c)
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria

Previous Serial Number: SAME

Principal Investigator: Donald P. Tschudy, M.D.

Other Investigators: C. Richard Magnussen, M.D.

Cooperating Units: Mathematical Biology Branch

Man Years

Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: To study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor-host relationships as well as primary diseases of porphyrin metabolism.

Methods Employed: Quantitative enzyme determinations, chemical determination of porphyrins and porphyrin precursors, use of tissue preparations and isotope methods in studying metabolic pathways and computer solutions of certain mathematical models.

Major Findings: A method has been developed for the measurement of uroporphyrinogen I synthetase in red cells. The optimal conditions for measurement of the enzyme have been worked out in detail and the method gives results which are highly reproducible. This enzyme is decreased in the liver in patients with acute intermittent porphyria (AIP) and is thought to be the fundamental defect in this disease. The mean value of the enzyme level in the red cells of 9 patients with AIP was close to 1/2 the mean value in 28 normals. This indicates that the mutation results in complete loss of catalytic activity. The range of red cell enzyme values in patients with AIP did not overlap the normal range. In 10 children with a parent known to have AIP, 5 were in the normal range, 4 were in the "porphyric" range and one was indeterminate. This is the expected frequency. The findings indicate that the red cell enzyme test is of great value in detecting patients with AIP. Since the children who were studied had urines which were "negative for porphyria" the data in these individuals suggest the enzyme test to be superior to chemical studies of the urine in the detection of AIP. Furthermore the fact that patients with other types of porphyria had normal red cell levels of uroporphyrinogen I synthetase, indicates that the test is capable of distinguishing AIP from other types of porphyria.

Previous studies in this laboratory suggested the presence of an excess of a substance in tumors which blocks the induction of hepatic ALA synthetase. An assay system for this substance using chick embryo liver has been developed and preliminary studies of tumor fractions are in progress.

A mathematical model for the control of enzyme synthesis has been developed. It utilizes a closed negative feedback loop in a chemical system. The non-linear differential equations which describe the kinetic behavior of the components of the loop cannot be solved analytically. In collaboration with Drs. Berman and Millar numerical solution of these equations has been achieved on a computer using the SAAM program developed by Dr. Berman. The control loop demonstrates a high degree of stability of enzyme level, but oscillatory activity can be produced by certain perturbations of the system.

Significance to Biomedical Research: Mechanisms of the control of metabolic pathways and the synthesis of enzymes in normal and diseased tissues are being elucidated. Some of the findings have been used for practical application to the diagnosis and treatment of certain types of porphyria. New abnormalities of control mechanisms in tumors and host tissues have been demonstrated and are under current study.

Proposed Course: (A) To continue studies of the factors controlling heme and heme-enzyme synthesis in normal and pathologic tissues. (B) To continue studies of biochemical abnormalities of porphyria in man and experimental animals. (C) To study control mechanisms of heme synthesis in tumors and livers of tumor-bearing hosts.

Honors and Awards

None

Publications

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Serial No. NCI-4004(c)
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Amino Acid Transport and Amino Acid Biosynthesis

Previous Serial Number: SAME

Principal Investigator: James M. Phang, M.D.

Other Investigators: S. Peter Nissley, M.D., David L. Valle, M.D. and Edward Kowaloff, M.D.

Cooperating Units: None

Man Years:

Total: 6
Professional: 2
Others: 4

Project Description:

Objectives: The biosynthesis, degradation and transport of amino acids play an important role in cellular growth and metabolism. The mechanisms by which these processes are regulated in normal and malignant cells are poorly understood. This project emphasizes the regulatory interplay in amino acid metabolism. It is relevant to Objective 3, Approaches 1 and 5 of the National Cancer Program.

Methods Employed: We are primarily using animal cells maintained in long-term tissue culture. Cells are cloned to insure a genetically homogenous population. Mutants are isolated by standard techniques. A variety of biochemical techniques have been utilized to study amino acid transport processes and amino acid biosynthesis in cultured cells. Uptake of amino acids may be assessed by using labeled L-amino acids and rates of protein and collagen synthesis can be obtained by incubating cells with L-proline-C^{14} and separation of the radioisotopically incorporated amino acids as proline and hydroxyproline by thin layer chromatography. The enzymes for biosynthesis and degradation of proline are assayed by using appropriate labeled precursors and quantitating the products isolated by ion exchange chromatography. Our methods are sensitive enough to measure these enzymes in leucocytes isolated from 10 ml of blood.

Major Findings: In mammalian cells the committed step of proline biosynthesis is catalyzed by the enzyme, Δ^1-pyrroline-5-carboxylate reductase which reduces Δ^1-pyrroline-5-carboxylate (PC) to proline. PC, on the other hand, is the product of the reaction catalyzed by ornithine-δ-transaminase which transaminates ornithine and α-ketoglutarate. PC may also be produced from glutamic acid although the enzymatic steps remain undefined in mammalian cells. The proline degradative pathway includes oxidation of proline to PC by proline oxidase and conversion of PC to glutamate by PC dehydrogenase.

Phenotypic Alterations in PC Reductase. Regulation of amino acid biosynthetic enzymes in mammalian cells remains poorly understood. Our studies show that PC reductase, the enzyme catalyzing the committed step in proline biosynthesis is regulated by a novel mechanism. We previously reported PC reductase was sensitive to proline feedback inhibition in rapidly growing cultured cells but was insensitive to proline inhibition in tissues. We have now shown that in resting cells undergoing growth activation, PC reductase is converted from the proline insensitive to the proline sensitive form. Resting lymphocytes have a PC reductase which is inhibited only 20-30% by 10^{-3}M proline. After 72 hours of incubation with phytohemagglutinin, a well known activator of lymphocytes, show a 15-fold increase in PC reductase. Of greater interest, the enzyme becomes 90% inhibited by 10^{-3}M proline. Over the 72-hour period the increase in PC reductase parallels the appearance of proline sensitive enzyme. Cycloheximide or actinomycin D will block both the increase in activity and the appearance of proline sensitivity. These findings suggest that activation of resting cells includes the expression of new genes producing a phenotypically altered PC reductase. In activated cells, the increased activity of the enzyme allows for maximal proline synthesis while the sensitivity of the enzyme to proline inhibition spares precursors vital for other cellular functions.

Type II - Hyperprolinemia - PC Dehydrogenase Defect. Patients with Type II hyperprolinemia are characterized by a high plasma proline concentration (10 x normal) and overflow iminoglycinuria. In addition, they excrete in the urine large amounts of O-aminobenzaldehyde reactive material, presumably PC. Fibroblasts were obtained from two unrelated patients with Type II hyperprolinemia. Both had undetectable PC dehydrogenase activity as compared to normal activities found in fibroblasts of 4 normal subjects. By contrast, the activities of ornithine-δ-transaminase and PC reductase activities were the same in Type II hyperprolinemia fibroblasts as in normals. This metabolic defect can also be diagnosed in circulating leucocytes. We obtained leucocytes from one patient with Type II hyperprolinemia, her parents and three siblings. As compared to leucocytes from 10 normal subjects (PC dehydrogenase activity, 40 ± 2 nMoles/hr/mg protein) the propositus had undetectable levels; mother, 10 nMoles/hr/mg protein; father 25 nMoles/hr/mg protein and the three siblings, 23, 23, and 21 nMoles/hr/mg protein.

Viral Transformation. Proline oxidase, a hepatic mitochondrial enzyme converting proline to Δ^1-pyrroline-5-carboxylate, is one of the enzymes involved in gluconeogenesis. In cultured mammalian fibroblasts proline oxidase activity is undetectable throughout the growth cycle.

In SV40 transformed fibroblasts, however, significant enzyme activity can be measured in both whole homogenates and isolated mitochondria. The activity of ornithine-δ-transaminase, another mitochondrial enzyme, is identical in normal and SV40 transformed fibroblasts. We are studying this enzyme in several other transformed fibroblast lines to ascertain if the presence of proline oxidase is a marker enzyme for transformation.

Significance to Biomedical Research: Cell membranes may play an important role in the regulation of cellular physiology. Changes in cell membrane function affects cellular metabolism and cell proliferation. Some of these changes are produced by hormone regulation as a component of normal control mechanisms. Abnormal changes in membrane function may result in uncontrolled neoplastic cell growth. Investigators have proposed that one of the primary changes produced by malignant transformation may be alterations in membrane function. These workers have shown that a viral transformation of cells can increase rates of sugar and amino acid transport even prior to increases in cell growth rates. Our studies on the regulation of amino acid transport and the interplay between membranes and endogenous biosynthetic mechanisms may elucidate the biochemical mechanisms producing derangements of these functions in malignant cells.

Proposed Course: The phenotypic alterations in pyrroline-5-carboxylate reductase would be of greater significance if the phenomenon can be generalized to other cell types. We are conducting experiments in an attempt to show that other cultured cells and tissues undergo this alteration of PC reductase concommitant to growth activation. We are also attempting to identify and purify the putative isozymes from resting and activated cells to prove that the isozymes are products specified by different genes.

The depression of specific enzymes with viral or chemical transformation is of considerable interest. Since many of the properties of transformed cells may be related to the intracellular concentrations of cyclic AMP and cyclic GMP, we are studying the effects of these cyclic nucleotides on the enzymes of proline metabolism. We are also measuring the levels of cyclic nucleotides to correlate changes in enzyme activity to changes in levels of cyclic nucleotides. The identification of patients with specific enzyme defects in the proline metabolic pathway will be pursued. In addition, cultured cells from these patients will allow us to study changes in the other enzymes of the pathway secondary to the metabolic block.

Honors and Awards

None

Publications

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Serial No. NCI-4014(c)
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of the Immune Response in Normal and Pathological States

Previous Serial Number: SAME

Principal Investigator: Warren Strober, M.D.

Other Investigators: T.W. Waldmann, R.M. Gebhard, G.N. Rogentine, D.L. Nelson, and H. Kleaveman

Cooperating Units: Digestive and Hereditary Diseases Branch, NIAMDD

Man Years:

Total: 5
Professional: 3
Other: 0

Project Description:

Objectives: Studies were directed toward determining the nature and mechanism of circulating and cellular immune responses to antigenic challenges in animals and humans, including patients with abnormal immunologic responses such as gluten-sensitive enteropathy, dermatitis herpetiformis (DH), and inflammatory bowel disease. In addition, studies were directed to determining the mechanism of disease in various immunodeficiency states such as ataxia telangiectasia, hypogammaglobulinemia and intestinal lymphangiectasia. Insight into the cause of the high incidence of reticuloendothelial malignancies associated with these disorders was sought.

Methods Employed: A host of techniques were used to assess immunological function of patients and experimental animals. Circulating B-cells from peripheral blood and tissue were enumerated with immunofluorescent immunoglobulins. Circulating and tissue T-cells were enumerated with sheep red blood cell rosette techniques. Antibody responses was assessed with hemagglutination techniques. *In vitro* proliferative responses (micro and macro) were assessed with tritiated thymidine incorporation following exposure to specific and nonspecific mitogens. The presence of mitogen-induced cytotoxic effector cells was assessed with a ^{51}Chromium release assay, using red cell and epithelial cell targets. The presence of antibody-mediated cytotoxic effector cells was assessed with ^{51}Chromium release assays using red cell and epithelial cell targets in the presence of appropriate antibody. Short term (1-2 hour) organ culture was used in conjunction with a solid phase immunoabsorption techniques to measure local mucosal immunoglobulin synthesis. Long term (24-48 hour) organ culture was used to develop in vitro models of intestinal disease. In addition to these specialized techniques, patients with various immunologic disorders were studied with immunoelectrophoresis, radial immunodiffusion, and metabolic turnover techniques for measurement of immunoglobulin synthesis and catabolism.

Major Findings: The functional capacity of organized gut-associated lymphocytes (Peyer's patch lymphocytes) was initiated. It was shown that in common with other lymphoid tissue, guinea pig Peyer's patches contained cells which bear a receptor for sheep erythrocytes. These cells undergo proliferation when exposed to a variety of nonspecific mitogens, including Conconavalin A, PHA and pokeweed mitogen. In addition, these cells can be induced by these mitogens to become cytotoxic effector cells with activity against various erythrocyte and epithelial cell targets. These studies indicate that Peyer's patches contain functional T-cells with cytotoxic capability. In other studies, it was shown that lymphocytes from Peyer's patches bear receptors for aggregated guinea pig gammaglobulin. However, unlike other cell populations also contain cells bearing aggregate-receptors, Peyer's patches cells cannot be enlished by specific antibody to become cytotoxic effector cells. Since antibody-mediated cellular cytotoxicity is at least in part a B-cell function, these results may be due to the fact that B-cells within Peyer's patches are functionally immature and/or deficient. In this sense they are similar to the cells observed in B-cell leukemias which also cannot participate in antibody-mediated, cellular cytotoxicity, but bear aggregate-receptors.

Studies on the pathogenesis of gluten-sensitive enteropathy were continued. Gluten-sensitive enteropathy is a disease of the GI tract associated with malabsorption, villous atrophy, and an increased incidence of neoplasia. We have shown that patients with this disease produce antibody with gluten specificity at mucosal sites following exposure to dietary gluten. In the current series of experiments the relationship of this finding to the pathogenesis of the disease was investigated. First, we noted that in organ cultures of jejunal biopsy tissue, brush border alkaline phosphatase activity increased, probably as a result of cell maturation. In tissues of patients with active gluten-sensitive enteropathy initial alkaline phosphatase activity was greatly diminished, but increases were seen during the 48 hours of culture. However, when the jejunal biopsy tissue was cultured in the presence of gluten-peptides, these increases in alkaline phosphatase activity during culture were greatly inhibited. The organ culture of jejunal biopsy tissue in patients with GSE in the presence or absence of gluten-peptides is an in vitro model of GSE since 1) the tissue manifests injury, i.e., a low alkaline phosphatase activity; 2) improves in the absence of gluten-peptides, i.e., undergoes an increase in alkaline phosphatase activity; and 3) fails to improve in the presence of gluten-peptides.

Whereas gluten-peptides affect cell maturation (alkaline phosphatase increase) during culture of tissue from patients with active disease, they have a greatly diminished effect on cell maturation in tissue from patients with inactive disease (patients on a gluten-free diet). This suggests that gluten is not directly toxic to the GI mucosa of GSE patients but requires the activation of an endogenous mechanism. To substantiate this possibility, tissue from patients with active disease was cultured together with tissue from patients with inactive disease in the presence and absence of gluten-peptides. It was observed that although gluten-peptides had no effect on inactive tissue culture alone,(alkaline phosphatase increased during culture) it prevented maturation of inactive tissue cultured in the presence of active tissue, (alkaline phosphatase increases were inhibited during culture). Control mixture experiments using normal tissue in place of active tissue did not have similar effect on the inactive GSE tissue. These experiments show that tissue from patients with active GSE elaborates a soluble factor which, in concert with gluten-peptides, prevents tissue maturation in GSE tissue. It is reasonable to assume that this soluble factor is antibody with gluten specificity.

In other studies the relationship between histocompatability type and gluten-sensitive disease was further explored. It had been found earlier that in dermatitis herpetiformis, a skin disease associated with gluten-sensitive enteropathy in many instances, the histocompatability antigen, HL-A8, occurred at a greatly increased frequency. In the present series of studies, it was shown that in two separate populations HL-A8 is especially frequent (80-90% incidence) in those patients with DH who have gastrointestinal disease and infrequent in those patients with DH who do not have gluten-sensitive gastrointestinal disease (20% incidence). In one of the patient populations studied, patients with DH and gastrointestinal lesions were identified by both morphologic criteria and local IgA synthetic abnormality. This allowed a more complete distinction between non-gluten and gluten-sensitive DH patients. This finding links HL-A8 more closely with the gastrointestinal lesion of gluten-sensitive enteropathy and implies that the gene responsible for HL-A8 acts in cooperation with other genes to produce gluten-sensitive disease.

The relationship between HL-A8 and gluten-sensitive disease was also studied in organ culture experiments. It was observed that gluten-peptides prevent tissue maturation in biopsy tissue of active patients in organ culture only if such tissue was obtained from patients with histocompatability antigen HL-A8. If tissue from active patients not bearing HL-A8 was studied, gluten-peptides had no effect on tissue maturation in culture. This finding is probably related to subtle differences in disease mechanisms of HL-A8 positive and HL-A8 negative individuals with GSE .

In further studies of dermatitis herpetiformis the gluten-sensitive gastrointestinal lesion was studied using organ culture techniques. It was found that in contrast to the patients with ordinary gluten-sensitive enteropathy, gluten-peptides did not prevent an increase in alkaline phosphatase activity during culture of dermatitis herpetiformis jejunal biopsy specimens, even when taken from patients with quite marked gastrointestinal lesions and whether or not the patient or the HL-A8 antigen on their leucocyte surfaces. This constitutes a subtle but important distinction between the two diseases and may be related to differences in binding affinity of the gluten-peptide to epithelial surfaces in DH and gluten-sensitive enteropathy.

Chronic ulcerative jejunitis is a ulcerative lesion of the small bowel associated with villous atrophy. Although an antecedent history of gluten-sensitivity is found in most cases, during the ulcerative period of disease patients are not gluten responsive. A patient with chronic ulcerative jejunitis was studied using techniques developed in this laboratory. It was found that the patient had a high jejunal IgA synthetic rate whether on a gluten-free or a gluten-containing diet; this contrasts with ordinary GSE patients whose synthetic rates fall following gluten restriction. In organ culture jejunal tissue from the patient during a period of gluten restriction behaved as does tissue from patients with GSE in remission: alkaline phosphatase increased were unaffected by gluten -peptides in the culture medium; similarly following dietary gluten challenge, jejunal tissue from the patient behaved as does tissue from patients with GSE in exacerbation; alkaline phosphatase increases were inhibited by gluten-peptides in the culture. We have concluded that chronic ulcerative jejunitis is a gluten-sensitive disease associated with a super imposed disease process, probably immunologic in nature, which results in continued villous atrophy and ulceration in spite of dietary gluten restriction.

Patients with gluten-sensitive enteropathy were studied for lymphocyte mitogenic responses to nonspecific mitogens and to soluble gluten-peptides or gluten-peptides linked to sepharose beads. Patients gave normal proliferative responses to nonspecific mitogens and no responses to gliadin in either of the two physical forms. These studies show that patients with gluten-sensitive enteropathy have normal delayed hypersensitivity as measured by in vitro responses. In addition, they mitigate against the immune response gene hypothesis of the disease inasmuch as one would expect positive lymphocyte proliferative responses in culture when lymphocytes are exposed to gluten antigen if an immune response were present.

Studies were initiated on the immunologic abnormality in inflammatory bowel disease. Peripheral lymphocytes of patients were evaluated by proliferative responses, by capacity to enter mitogen-induced cytotoxicity and by studies of profiles of peripheral T-and B-cell populations. Using these parameters, no abnormalities of immunologic function in patients with inflammatory bowel disease was found.

Significance to Biomedical Research: Studies of patients with gluten-sensitive enteropathy have provided new insights into the operation of the local mucosal immunologic system. They suggest that local responses may be under the control of immune response genes, or alternatively, they depend on the presence or absence of binding (receptors) on the surface of gut epithelial cells. Each of these possibilities are compatable with and are indeed suggested by the observed high frequency of HL-A8 in gluten-sensitive disease. In the first instance, HL-A8 may be linked to an abnormal immune response gene which leads to the production of pathogenic antigluten antibodies. Alternatively, these genes may be responsible for the binding protein or may be linked to another gene responsible for a finding protein on the surface of gastrointestinal epithelial cells. If the latter possibility is true it may indicate that antigens entering the GI tract are more immunogenic if they become attached to the epithelial cell surface and thereby mimic antigens arrayed on particulate organisms.

Studies of the immunology of the GI tract, particularly the organized gut associated lymphoid tissue in Peyer's patches may lead to an increased insight into the mechanism whereby the GI tract contains the normal bacterial flora of the bowel. As such, these studies can lead to an understanding of the relationship between normal cell proliferative processes in the GI tract and the abnormal cell proliferative processes observed in GI neoplasms.

It is well known that patients with gluten-sensitive enteropathy, DH and inflammatory bowel disease as well as patients with immunodeficiency are subject to a greatly increased risk of neoplasia. A variety of evidence points to the immunologic system as an important bulwark against the development of neoplasia. Studies of the diseases characterized by immunologic system acts to prevent neoplasia as well as how aberrant immune responses may predispose to neoplasia.

Proposed Course: Continued studies of the role of immunologic factors in gluten-sensitive enteropathy and other gastrointestinal diseases will be carried out. In particular, the organ culture system will be utilized to show that antibodies with specificity for gluten protein are an essential component in the pathogenesis of gluten-sensitive enteropathy. This demonstration is now possible using the in vitro model of gluten-sensitive enteropathy that has been developed in the laboratory. It is proposed that patients with other gastrointestinal diseases will be studied with similar techniques. In particular, patients with inflammatory bowel disease will be evaluated using standard immunologic evaluation studies as well as studied of B- and T-cell populations. In addition, patients with inflammatory bowel disease will be studied using both mitogen-induced and antibody-mediated cytotoxicity assays.

In the area of gastrointestinal immunology the nature of cells making up gut associated lymphoid tissue will be intensively investigated. In particular, the full functional capabilities of Peyer's patch cells will be examined. Finally, the ability of Peyer's patch cells to producing immunoglobulins to specific and nonspecific mitogens will be studied using newly developed techniques of assaying immunoglobulin synthesis in vitro.

Honors and Awards

None

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1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of the Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense

Previous Serial Number: SAME

Principal Investigator: R. Michael Blaese, M.D.

Other Investigators: Thomas Waldmann, M.D., Warren Strober, M.D., J.J. Oppenheim, M.D., J. Wunderlich, M.D., L. Altman, M.D., A. Muchmore, M.D., S. Rosenoff, M.D., R. Young, M.D., H. Kirchner, M.D. and D.L. Nelson, M.D.

Cooperating Units: Immunology Branch, NCI, and Laboratory of Microbiology, Immunology Section, NIDR, and Medicine Branch, NCI.

Man Years:

Total: 4
Professional: 2
Other: 2

Project Description:

Objectives: The objectives of this study were to determine mechanisms important to the development and function of both humoral and cellular immune response in animals and man; to determine the nature of the deficiency in immune function characterizing such disease states as the Wiskott-Aldrich syndrome, intestinal lymphangiectasia and advanced malignancy; and, to develop approaches to prophylaxis and/or therapy of the infectious and neoplastic disease frequently associated with defective immune processes. This work is directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: Antibody responses of experimental animals and man vere determined after immunization with bacterial vaccines such as brucella abortus, S. typhosa, and pertussis; with particulate cellular antigens such as sheep, burro, and chicken RBC's; with purified protein antigens such as KLH, diphtheria and tetanus toxoids, guinea pig-γ-globulin and bovine γ-globulin; with purified polysaccharide antigens such as pneumococcal polysaccharides types I, II, III, and the V_i antigen of E. coli. The antibodies were detected by standard techniques or by newly developed micro methods of bacterial agglutination, red cell hemolysis, and passive hemagglutination using $CrCl_3$ as an agent to couple the antigens to a carrier RBC surface. In addition, modifications of the Farr antigen binding assay were used.

Cellular immune responses were measured by delayed hypersensitivity skin tests, contact sensitivity to DNCB, and skin allografts. Lymphocyte transformation in vitro was carried out utilizing non-specific mitogens such as PHA, PWM and staph filtrate; specific antigens such as SLO, SKSD, diphtheria and tetanus toxoids, KLH and candida albicans; and allogeneic cells in mixed leukocyte culture. Lymphocyte function in vitro was also measured by production of mediators of cellular immunity such as lymphotoxin, MIF, and lymphocyte derived chemotactic factor for monocytes (LDCF) and by cytotoxicity reactions with antibody sensitized target cells or with non-specific mitogen activated effector lymphocytes.

Studies of the characteristics of various cell surface receptors on immunocompetent lymphocytes and monocytes-macrophages utilized autoradiography, immunofluorescence microscopy, and cellular rosette formation.

Major Findings: Study of patients with immunodeficiency disease and a high incidence of malignancy was continued with the ultimate goal of correction of the immunologic defect in these patients and gaining insights into the role of immune processes in prevention and control of malignant disease.

The Wiskott-Aldrich syndrome an x-linked immunodeficiency disorder, has a very high incidence of infections with all classes of microorganisms and, in addition, the highest rate of malignant disease of any of the immunodeficiency diseases. Intensive study of these patients has demonstrated pronounced defects in both humoral and cellular immunity characterized by clinical anergy and defective antibody production. In spite of these pronounced clinical immune defects, our studies have demonstrated that much of the effector arm of their immune system is intact. They have normal numbers of circulating T and B lymphocytes as detected by immunofluorescence or microcytotoxicity assays with specific antisera. They produce immunoglobulins at normal or elevated rates. Their lymphocytes have normal proliferative responses in vitro to non-specific mitogens and they produce lymphokines such as macrophage migration inhibitory factor (MIF), lymphotoxin and chemotactic factor for monocytes normally. In addition, the killer function of their B lymphocytes is normal as detected by in vitro antibody directed lymphocyte mediated cytotoxicity, and their T lymphocyte killer cells are normal as tested in PHA activated killing of ^{51}Cr-labeled target cells in vitro. In spite of these normal in vitro tests these patients lack adequate specific humoral and cellular immune responses both in vitro and in vivo which supports our view that the immunodeficiency of this disorder is the result of a defect in afferent limb of immunity, perhaps a defect in antigen recognition or processing or representing defective control of their immune responses. Defective control of immunoresponsiveness is also suggested by the observation that many of these patients have developed transient monoclonal gammopathy. In addition, the unstimulated lymphocytes from these patients in vitro were found to spontaneously produce as much of a chemotactic factor for monocytes as do lymphocytes from normal individuals only after antigen or mitogen stimulation. This high level of unstimulated chemotactic factor production apparently contributes to the anergy in these patients by producing high circulating levels of chemotactic factor which tends to abolish any chemotactic gradient produced by a specific immune response and also by deactivating the chemotactic responsiveness of the monocytes from these patients.

A most striking feature of the WAS is the very high incidence of lymphoreticular malignancy found in these patients. Six of the 13 patients studied at the NIH since 1966 have developed malignancy. In a very provocative study in collaboration with Drs. K.K. Tokomoto and A.S. Robson, we have isolated a papovavirus from a reticulum cell sarcoma involving the brain of one of our patients. Serum from this patient was studied for antibody to the virus and about 14 months prior to the clinical appearance of the tumor the patient developed a striking antibody titer to this virus. Following the isolation of this papovavirus from the child's brain tumor, the virus was also isolated from his urine on each of 3 attempts. At post mortem examination, 5 months after the initial brain tumor virus isolation, the patient was found to have massive tumorous invasion of the kidneys, perhaps explaining the presence of virus in the urine. The isolation of a virus belonging to the papova group, which are well known for their oncogenic properties, from a human tumor raises intreging questions in regard to the possible role of these viruses in human cancer. Although this single observation certainly does not prove an etiologic relationship between this virus and cancer, it does provide a strong stimulus to further investigation of its association with malignancy, particularly that occurring in immunodeficient individuals.

Studies of several aspects of cell mediated immunity were continued in patients with a variety of neoplastic or immunodeficiency diseases and in basic animal models. In vitro cellular immune function has been assessed by four basically different techniques: 1. The proliferative response of lymphocytes following stimulation with mitogens, antigens, and allogenic cells; 2. the production of biologically active soluble mediators (lymphokines) secreted by stimulated lymphocytes; 3. the cytotoxic-killer activity of both immune and nonimmune lymphoid cells; and 4. the in vitro responsiveness of mononuclear leukocytes (monocytes and macrophages) to various lymphokines and other biological mediators such as activated serum complement components.

Studies of patients with intestinal lymphangiectasia have demonstrated profoundly impaired peripheral blood lymphocyte proliferative responses in vitro to all classes of mitogenic stimuli. Intestinal lymphangiectasia is a disease characterized by a disorder of lymphatic channel development leading to dilitation of the lymphatic channels of the small bowel, the hallmark morphologic lesion of this disease, associated with bulk loss of lymphatic fluid into the GI tract. We have postulated that a qualitative defect in the lymphocyte pool of these patients results from the selective depletion of the long lived recirculating T-lymphocytes by gastrointestinal loss. If the postulate is true, then reversal of the gastrointestinal lymph loss should result in reversal of the immunodeficiency in these patients. We have recently had the opportunity to test this hypothesis directly in a patient whose intestinal lymphangiectasia was secondary to constrictive pericarditis. This patient had profound hypoproteinemia and lymphocytopenia with marked clinical cellular immunodeficiency as determined by failure to reject a skin allograft and inability to mount any delayed type hypersensitivity reaction. Following successful pericardectomy the patient's gastrointestinal protein loss ceased, his peripheral lymphocyte count returned to normal, he rejected skin allograft and developed positive delayed hypersensitivity reactions to many common environmental antigens, and the *in vitro* reactivity of his peripheral lymphocytes returned to normal. Thus, our experience with this patient directly demonstrates that the entire immunodeficiency syndrome associated with intestinal lymphangiectasia can by accounted for by the loss of immune effectors into the gastrointestinal tract.

Patients with chronic lymphocytic leukemia also have profound defects in *in vitro* lymphocyte function and frequently have clinical anergy as well. Peripheral blood lymphocytes from CLL patients generally fail to proliferate in response to mitogens, antigens or allogeneic cells. They also are unable to produce biologically active lymphokines and their lymphocytes are ineffective as killer cells in a variety of *in vitro* cytotoxicity assays. The predominant circulating lymphocyte in these patients is a B cell bearing homogenous 8S IgM on their surface. To determine if the profound *in vitro* deficiency of cells from these patients reflected a general lack of immunocompetent lymphocytes or alternatively, dilution of normal cells by a great excess of leukemia nonfunctional cells, normal T lymphocytes were isolated from the blood of these patients by their ability to form spontaneous rosettes with SRBC. Isolated T cells from these patients were found to respond almost normally in each of the assays which showed profound deficiency when their unseparated lymphocytes were tested. Thus, dilution of normally reactive lymphocytes with nonfunctional leukemia B cells appears to account for the general failure of CLL cells to proliferate, produce lymphokines, and to behave as cytotoxic effector cells in *vitro*.

Patients with Hodgkin's and non-Hodgkin's lymphoma have a variable degree of *in vivo* and *in vitro* immunodeficiency. Patients with the most advanced disease were found to have deminished numbers of circulating T lymphocytes and decreased in vitro proliferative responses, particularly when stimulated with specific antigens *in vitro*. Killer lymphocyte function in many of these patients was also defective while their ability to produce a lymphokine chemotactic for monocytes was generally in tact. The responsiveness of monocytes from these patients to chemotactic factors was also frequently abnormal. During the course of therapy, those patients having a beneficial response showed remarkable recovery of *in vitro* cellular immune function, while those patients whe were treatment failures also failed to develop improved cellular immune function in vitro. It thus appears that study of in vitro lymphocyte function may provide a useful prognostic index for the immediate prospects of remission in these lymphoid malignancies, although only longer periods of follow-up will indicate the long term prognostic implications of these observations.

An important in vitro correlate of cellular immune function is the ability of lymphocytes to kill various target tissues and cells, a role thought to be particularly important for host resistance to malignant transferred cells. To understand more about the types of lymphoid cells involved in these reactions and their mechanism of action, studies of these killer cell activities were carried out in several basic animal model systems.

Certain classes of antibody have the property that they induce non-immune lymphocytes to attack and kill target cells coated with these antibodies. These antibodies have been termed lymphocyte dependent antibodies (LDA) because their biologic activity depends upon lymphocyte action just as complement dependent antibodies require complement for their biologic activity. A question of central importance in the understanding of the mechanism of this form of lymphoid cell killer activity is the determination of the type of cell doing the killing and its characteristics. Studies of patients with a variety of T cell defects failed to disclose a consistent pattern of reactivity. However, patients with intestinal lymphangiectasia were found to have significantly enhanced LDA killer cell activity in their peripheral blood and depressed killer activity by the lymphocytes isolated from their chylous effusions. This differential killer activity of these two lymphocyte pools was exactly the opposite of the in vitro proliferative activity which we had previously observed in these same lymphocyte pools suggesting that LDA killer cells were predominantly from the non-recirculating lymphocyte population whereas the lymphocytes responding with blastogenic transformation were from the long lived recirculating population. Further studies of LDA killer activity by lymphocytes from patients with hypogammaglobulinemia usually showed a markedly depression in this function while the lymphocyte proliferative responses from these patients were more nearly normal. However, certain hypogammaglobulinemic patients did have normal LDA killer activity. To resolve this apparent paradox, the cell surface receptor markers on B lymphocytes were compared with the degree of LDA killer activity observed. It was found that those hypogammaglobulinemic patients who lacked both immunoglobulin and receptors for IgG-Fc on their lymphocytes had the most profound depression in LDA killer activity, while those hypogammaglobulinemic patients whose lymphocytes had a normal compliment of IgG-Fc receptors killed normally. Thus, the LDA killer cell activity in man appears to reside in the non-recirculating B lymphocyte population and appears to be dependent upon a normal IgG-Fc receptor on these cells. It was also observed that antigen-antibody complexes or aggregated IgG (with subsequently activated Fc components) could effectively block the killer activity of these cells, presumably by competitively blocking the Fc receptor site on these killer cells.

Another in vitro test for competence of cell mediated killing has been developed from the observation that normal nonimmune lymphocytes will attack and kill certain target tissues or cells if incubated together in the presence of phytomitogens like PHA. The killing observed in this system occurs very rapidly and therefore is apparently measuring the cytotoxic potential of a pre-existing killer cell rather than causing the generation of new killers. It was also found that the killing observed in this system was dependent on a direct link being formed between the killer cell and the target via the mitogen. Thus, although concanavalin A was capable of inducing lymphocytes to kill burro red cells, it was ineffective in inducing lysis of human red cells. Conversly, pokeweed mitogen induced lymphocytes to kill human but not burro red cells. By appropriate mixture and absorption studies it was shown that both the killer cell and the target cell must each have receptors on their surfaces for the mitogen to allow a cross linking between the two, and that the killing observed could not be due to the release of a toxic lymphocyte mediator such as lymphotoxin. Since PHA and ConA are felt to be exclusively T cell mitogens, it was believed that the killer cell detected in these studies must, therefore, be a T lymphocyte. In studies utilizing lymphocytes from agammaglobulinemic chickens it was shown that those cells were just as effective as cells from normals, at least indicating that "B" lymphocytes are not required for this type of target cell killing. It was also found that cell suspensions depleted of all phagocytic cells were also effective killers so that monocytes, macrophages, and PMN's are also not required to obtain mitogen activated target cell killing. However, in recent studies it was also shown that spleen or bone marrow lymphocytes from athymic nude mice also can effectively kill these red cell targets in the presence of phytomitogens. Thus, cell populations depleted specifically of all three elements, T cells, B cells, and phagocytes - all retain mitogen induced killer cell activity suggesting that this form of cytotoxicity may reflect a much more undifferentiated function than previously supposed. In this regard, it is particularly interesting that a number of patients with immunodeficiency disease or malignancy do have severely depressed ability to kill targets via this mechanism, indicating that continued study of this phenomenon may disclose additional information concerning the pathophysiology of these disorders.

Studies of various factors influencing the development of immunocompetence in the neonatal period were continued. We have previously shown that neonatal rats acquire immune responsiveness to different antigens in a distinct sequence rather than simultaneous in early post-natal life. This apparent sequential maturation of immune competence was found not to be dependent upon stepwise differentiation of specific antigen reactive cells, but rather upon the maturation of components of the afferent limb of immunity. Macrophages play an essential role in the initiation of immune responses to many antigens, and it was demonstrated that immaturity of this system of cells accounted for much of the apparent immune deficiency in the neonate. Thus, when mature adult macrophages were given to the neonate, they produced antibody to a variety of antigens to which they were ordinarily unresponsive. It was also found that a variety of materials given to the neonates would result in accelerated maturation of their own macrophage population, and result in earlier immunocompetence. Thus, the adjuvant properties of such agents as endotoxin, BCG, alum, pertussis vaccine, silica, and poly A.U. were shown to function by macrophage activation. We have also shown that activation of the macrophage system of the neonate not only allowed antibody formation in otherwise unresponsive neonates, but also was effective in preventing the induction of specific immunological tolerance by high doses of antigen given at birth. In preliminary studies this finding was utilized to prevent tolerance to the vertically transmitted mammary tumor virus in C_3H mice and this non-specific immunoprophylaxis subsequently resulted in resistence of these mice to mammary tumor transplantation. Macrophage activation in neonatal C_{57} BL/6 mice, however, did not affect subsequent development of progressive tumors caused by neonatal infection with M-MSV.

Further studies of role of afferent limb were carried out to determine its effect on the cellular immune system. Adult macrophages and macrophage activating substances were found to enhance the ability of spleen cells from neonatal rats to induce a graft versus host reaction in F_1 hybrid recipients. Macrophage activation also promoted both the development of delayed type hypersensitivity and its expression by skin testing. Macrophage activation also significantly enhanced expression of cellular immunity to RNA virus associated tumor antigens in mice.

Significance to Biomedical Research: The present studies extend our understanding of the diverse, yet interrelated mechanisms contributing to the development and normal functioning of immune system in animals and man. They indicate that disorders of immune function may be related to the absence or malfunction of such central lymphoid organs as the thymus or bursa of Fabricius or to immaturity of such auxillary systems as the macrophage required for the processing of antigens in the initiation of an immune response. They also indicate that the macrophage plays multiple roles in a normal immune response, being both important in the initiation or afferent limb of such responses and also as the ultimate effector cell in many immune reactions. Our studies of lymphocyte function in patients with immune deficiency and advanced malignancy have extended our understanding of the nature of the reactions produced by different classes of lymphocytes and the observation that some of these functions may remain intact in severely anergic individuals may provide us with a mechanism of providing effective immunotherapy for malignancy occurring even in severely immune deficient patients. Similarly, the observation that the monocytes and macrophages are abnormal in some patients with cancer or immunodeficiency and that this defect may be corrected by transfer factor treatment offers another approach to utilize immune processes in therapy of disease.

Proposed Course: Continuing studies directed toward understanding the normal pathways involved in the development and function of the immune system and application of this new knowledge in approaches to the prevention and therapy of malignant disease.

Honors and Awards

None

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Smith, R.W., Blaese, R.M., Hathcock, K.S., and Edelson, R.L.: T and B lymphocyte markers in lymphoid cell research and in human disease. Proceedings of the 8th Leukocyte Culture Conference. In press.

Bruce, R.M. and Blaese, R.M.: Monoclonal gammopathy in the Wiskott-Aldrich syndrome. J. Ped. In press.

Blaese, R.M.: Macrophages and the development of immunocompetence. In Dayton, D. and Bellonti, J. (Eds.): The Phagocytic Cell in Host Resistance. In press.

Serial No. NCI-4016(c)
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanism of the Anabolic Action of Growth Hormone

Previous Serial Number: None

Principal Investigator: S. Peter Nissley, M.D.

Other Investigators: Kenneth L. Cohen, M.D.

Cooperating Units: None

Man Years:

Total: 3
Professional: 2
Other: 1

Project Description:

Objectives: The understanding of normal growth may be a prerequisite to understanding malignant growth. One experimental model for the study of normal growth has been the growth hormone treated hypophysectomized animal. It has been proposed that the anabolic action of growth hormone is mediated by a second circulating protein designated somatomedin. The object of this project is to purify somatomedin, develop an in vitro tissue culture system responsive to somatomedin and study the biochemical events accompanying growth in this system.

Methods Employed: Somatomedin is being purified from the conditioned medium of buffalo rat liver cells in culture. This rat liver cell line grows in the absence of serum and produces a growth factor identical to or closely related to somatomedin isolated from human serum.

Serum starved cells in culture are being utilized to study the action of the purified somatomedin. In order to establish whether or not growth hormone dependent factors play a role in the growth of cells in culture sera from normal, hypophysectomized and growth hormone treated hypophysectomized rats are being tested on cells in culture utilizing radioactive thymidine incorporation as a measure of DNA synthesis.

Major Findings: Tertiary cultures of chick embryo fibroblasts plated in 0.4% serum become synchronized in G-1 of the cell cycle after 48-72 hours. Addition of 1% normal rat serum results in a 10-fold increase in thymidine incorporation into trichloroacetic acid precipitable material after 12-14 hours. One percent hypophysectomized rat serum is only one-fifth as active as normal serum in this assay. Mixing experiments show that the decreased activity in the hypophysectomized serum is not due to an inhibitor. Treatment of hypophysectomized rats with bovine growth hormone restores 60% or more of the activity in the thymidine incorporation assay. The GH dependent increase in activity is heat stable and nondialysable. Bovine growth hormone is inactive in the assay and physiologic concentrations of insulin and cortisone show less than 5% of the activity of normal serum. Thus, chicken embryo fibroblasts which are known to be responsive (thymidine incorporation) to somatomedin isolated from the conditioned medium of buffalo rat liver cells, also respond in the expected way to sera from normal, hypophysectomized and growth hormone treated hypophysectomized animals.

Significance to Biomedical Research: The understanding of the biochemical events accompanying normal growth should provide a groundwork for understanding the aberations in growth accompanying malignancy. Also, further delineation of the hormonally dependent growth factors necessary for the growth of cells in culture is of general importance in tissue culture, a widely utilized technique in cancer research. Specifically this research supports objective 3, approaches 1 and 2 and objective 4, approach 1 of the National Cancer Plan.

Proposed Course: The studies with chick embryo fibroblasts utilizing sera from normal, hypophysectomized and growth hormone treated hypophysectomized rats will be extended to using sera from growth hormone deficient humans before and after growth hormone treatment. The somatomedin purified from the media of rat liver cells will be injected into rats to see whether or not the anabolic action of growth hormone can be duplicated in vivo. Studies are in progress to determine whether or not cyclic AMP and cyclic GMP are playing a role in the anabolic action of somatomedin in the tissue culture system.

Honors and Awards

None

Publications

None

SUMMARY REPORT
LABORATORY OF PATHOLOGY
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
July 1, 1973 to June 30, 1974

The diagnostic and research activities of the staff of the Laboratory of Pathology are listed by Sections. The research accomplishments are especially impressive when one considers that the majority of the staff participate to some extent in the diagnostic services for Clinical Center patients. In fact, because of changes in professional personnel, including resignations and retirement of some staff members, and because of additional administrative responsibilities of others, the overall diagnostic workload of several members of the staff has increased appreciably during the past year. This was particularly true for Ultrastructural Pathology, Surgical Pathology and Viral Oncology because the Head of each of these three sections resigned during the past year. Recruitments for an additional pathologist for each of these three sections have been successful and the workload will be more equitably distributed when these pathologists join these sections on July 1, 1974. It is by offering opportunities and facilities for laboratory research that we have been able to recruit and retain an outstanding group of anatomical pathologists who have given vital support to the clinical programs of NCI as well as all the other Institutes with patients in the Clinical Center.

The research programs of the Laboratory have involved:

A. Hematopathology and Immunopathology
B. Viral Oncology and Cell Biology
C. Comparative Pathology and Carcinogenesis
D. Development and use of Automated Equipment for Cytology Diagnosis
E. Clinico-pathologic Studies on a Variety of Human Diseases

Some of the significant research and clinico-pathologic accomplishments in which the staff of the Laboratory participated, either as principal investigators or as collaborators include:

1. Demonstration of the B cell origin of human nodular lymphomas of the poorly differentiated lymphocytic and mixed cell types using methods for demonstration of cell surface markers for B cells and the demonstration of monocyte-macrophage markers on the neoplastic cells of histiocytic medullary reticulosis.

2. Evidence that fiber size is critical in asbestos carcinogenesis.

3. Identification of initiation of viral protein synthesis as the site of the anti-viral action of interferon.

4. Ultrastructural demonstration of herpesvirus antigens on cell surfaces with hybrid antibodies.

5. Characterization of immune responses in animals infected with the oncogenic monkey herpesvirus, _H. saimiri_, and finding that antibodies to

early antigens of the virus are related to development of tumors.

6. Transformation of mouse salivary epithelium by polyoma virus in transfilter interaction.

7. Conclusion, from review of autopsy and surgical pathology material, that mycosis fungoides is a distinctive entity histopathologically and that its pattern of spread in lymph nodes and spleen mimics the normal migration of T-lymphocytes.

Many members of the staff, in addition to their laboratory and service work, serve on numerous national and international committees, editorial boards, etc. Some of the principal activities of this sort are: Dr. Powell, member of International Association for the study of Lung Cancer; Dr. O'Conor, Chairman of Epidemiology Committee of the Breast Cancer Task Force, Chairman of USA National Committee for UICC and Chairman of Commission on Epidemiology, UICC; Dr. O'Conor and Dr. Thomas, WHO Collaborating Centers for Classifications of Neoplasms; Dr. Herman, Chairman of Committee on Cytology Automation; Dr. Berard, Pathology Editor of the Journal of the Reticuloendothelial Society and Associate Editor of Laboratory Medicine; Dr. Stanton, Scientific Editor of the JNCI; Dr. Berard and Dr. Thomas, Pathology Panels for the study of Malignant Lymphomas and Mycosis Fungoides; Dr. Henson, Committee on Nomenclature and Classification of Disease, College of American Pathology. Other committees, symposia, etc., participated in by the staff are listed in their annual reports.

Dr. O'Conor during most of 1973 was on assignment in the Director's office. He resigned as Head of the Section of Surgical Pathology and was reassigned as Associate Director for International Research in November 1973. Dr. Powell was subsequently appointed Head of this Section and Dr. Jose Costa, currently a Visiting Fellow working with Dr. Rabson in Viral Oncology, will be reassigned to the Section on Surgical Pathology as a Senior Staff Fellow on July 1, 1974.

Dr. Grimley resigned as Head of Ultrastructural Pathology late in the Spring of 1973. Dr. Thomas has been Acting Head of the Section. Dr. Timothy J. Triche, a pathologist and electron microscopist from Washington University School of Medicine, St. Louis, will become a Senior Staff Fellow in Ultrastructural Pathology on July 1, 1974.

Dr. Raul C. Braylan started working in the Hematopathology Section as a Senior Staff Fellow on July 1, 1974. Dr. Elaine S. Jaffe who has worked with Dr. Berard in Hematopathology since July 1972 will become a permanent Staff Pathologist in the Section on July 1, 1974. Dr. Risa Mann was a Guest Worker in Hematopathology for approximately three months. Dr. Yamamoto was a Guest Worker supported by the Atomic Bomb Casualty Commission in Hiroshima and the Japan NIH from April 1973 to October 1973.

Dr. Friedman returned from his assignment in England in September 1973 and resigned in February 1974. Dr. Nubia Munoz was a Visiting Associate in Viral Oncology until October 1973. Dr. Nathaniel Young has been recruited and will join Dr. Rabson in the Section on Viral Oncology on July 1, 1974.

During the first six months of 1974, Dr. Hans Popper was a Fogarty Scholar and regularly participated in the teaching of residents and review of diagnostic materials in the Laboratory of Pathology. He and Dr. Thomas collaborated in a review of the hepatic lesions (angiosarcomas and hepatic fibrosis) of the workers exposed to vinyl chloride and polyvinyl-chloride.

Dr. Maila Penttinen, a Visiting Fellow, started working with Dr. Chu in the Cytopathology Section in July 1973.

The staff of the Laboratory of Pathology were either senior authors or co-authors of 56 papers and 8 abstracts published in 1973. Eleven papers and 2 abstracts have been published through March of 1974. Twenty-six manuscripts have been accepted for publication and are in press. (See attached list of publications).

Surgical Pathology Section:

Dr. O'Conor, until his reassignment, and Dr. Powell, together with the residents and other pathologists are responsible for the diagnostic services in Surgical Pathology. Approximately 6017 specimens or biopsies were accessioned. These included approximately 2703 bone marrows sectioned and processed in the Laboratory. Dr. Powell and the resident staff regularly make rounds with the staff of the Surgery Branch and show most of the surgical biopsies and operative specimens at the weekly Surgical-Pathology Conference. Other conferences based on surgical or biopsy material are conducted with clinical groups including the hematology-lymphoma conference and the lymphoma staging conference (Drs. Berard, Jaffe and Braylan); the liver conference (Dr. Jaffe and Dr. O'Conor); and the radiation therapy conference (Dr. Thomas).

Postmortem Section:

Dr. Thomas, the residents and other staff were responsible for 144 autopsy examinations. Ninety-four percent were performed on the 153 patients who died in the Clinical Center and 49 were on submitted slides and protocols of former Clinical Center patients who died elsewhere. A gross review of organs is held with the residents and clinical staff each morning at 8:30 a.m. and the staff and residents present selected autopsies at numerous clinicopathologic conferences. The program to remove eyes regularly has been successful and is continuing. Also many specimens of a variety of human tissues are regularly furnished to NIH scientists in various laboratories.

Publications of studies on human material:

Slightly more than half of the 1973 publications by the staff and residents were based on human tissues or some aspect of human disease. These include Hodgkin's disease and other malignant lymphomas (Berard 18,19,22,23,34,47,48; O'Conor 6,19,21,49,50; Thomas 13,35,47); occult metastases from cervical carcinoma (Rabson 1); production of human chorionic gonadotropin in cultured cells from a carcinoma of the lung (Rabson 4,14); culture line of human breast cancer (Chu 12); thyroid cancer (Henson 15); cancers among USA Indians (Dunham 16); tubulo-reticular structures in lymphocytes of L.E. patients (Grimley and Michelitch 24, Grimley and Schaff 43); surface IgE on basophils

(Grimley 28); bladder toxicity with cyclophosphamide (Chu 33); needle aspiration cytology (Chu 36); tumors of the respiratory tract (O'Conor 51); BCG immunotherapy (Powell 56); amyloidosis of the tracheo-bronchial tree (Powell 55); staining of Pneumocystis carinii (Bowling, Smith and Wescott 54); cultures of chondrocytes (Morgan 10); collagen of fibrous lung-spacing type (Banfield 11); and receptors for complement and immunoglobulin on lymphocytes (Jaffe 53). Reference numbers are to the 1973 list of publications.

Other Research Activities by Staff in Surgical Pathology and Postmortem Sections:

In addition to his diagnostic services and administrative work, Dr. O'Conor conducts research in electron microscopy. Included are published studies on the EM structure of a thymic lymphoma induced by antigenic stimulation and immunosuppression. He provides EM support for a number of collaborative projects including: studies on the effect of rifampin and Ara-C on CMV cultures and herpes virus aggregation by Con A (with Dr. Rabson); localization of gonadotropin in lung tumor cultures (with Dr. Rosen and Dr. Rabson); ultrastructure of retinoblastoma (with Dr. Albert) and neuroblastoma (with Dr. Costa); and regression of breast tumors (with Dr. Gullino).

Dr. Powell and the resident staff are studying a number of human diseases including: effects of chemotherapy and radiation therapy on bronchogenic carcinoma; changes in taste buds and olfactory apparatus in patients with disorders of taste and smell; review of 40 histiocytic lymphoma cases (Dr. Banks); spontaneous regression in a patient with metastatic melanoma in the liver (Banks); review of 28 cases of American Burkitt's lymphoma (Banks); review of 20+ cases of Wiskott-Aldrich syndrome (Witebsky); and changes in Meissner corpuscle in monkey finger skin following denervation (Witebsky). Dr. Thomas, in collaboration with Dr. H. Rappaport, has completed an extensive review of autopsy and surgical pathology material from patients with mycosis fungoides. A complete list of other human and experimental studies by the staff and residents is given in the individual annual reports.

Laboratory Services for Human Tissues:

Miss Bowling and her staff continue to provide excellent histological slides and special stains for human tissues. She also provides valuable consultation on special staining and preparation of human tissues to many clinical associates and other NIH scientists. During the year she published a rapid staining procedure for Pneumocystis carinii and is currently, in collaboration with Dr. Berard, preparing an extensive atlas on technical problems and artefacts encountered in processing tissues.

Photography services for human tissues are provided by Mr. Edwards and Mr. Banks, who in addition, maintains the slide files which contain more than a million slides. Immediate access to these and to the gross and microscopic photographs is invaluable for patient diagnosis and follow-up and for an increasing number of clinico-pathologic studies.

athology data processing has essentially stopped because we could not replace he secretary who was using the DCRT terminal for in-put of pathology diagnoses. ecently Dr. Henson attempted to develop a contract for this data processing ut we were informed that it would be illegal to use the contract procedure or in-put of pathology diagnoses from the DCRT terminal in the Laboratory.

ytopathology Section:

r. Chu and the staff are responsible for all diagnostic reports on exfolia- ive cytology specimens which include sputum, urine, cervical-vaginal smears, NS fluid, aspirated material, etc. In addition she and her staff are re- ponsible for examining materials for karyotyping and chromosome abnormalities. pproximately 5038 accessions (11913 smears) and 221 chromosome analysis pecimens (2210 smears) were processed and examined in 1973. This workload s about 20% greater than the workload in 1972. The evaluation of fine needle spiration for cytologic interpretation is continuing in collaboration with taff in the Surgery Branch. Other studies now in progress include study of reast carcinoma cultures by "G" banding technique, chromosomal characteristics f lymphoma cells, and the effects of oral Enovid on cervical and vaginal epi- helium of mice. Dr. Penttinen is working on the in-vitro production of olypoid cells with cytochalasin.

utomation of Cytology:

r. Herman and his staff made a great deal of progress on this project. The ultiparameter cell sorter was delivered from the Los Alamos Scientific Lab- ratory in November, 1973. It is now being used to investigate cellular haracteristics of normal, atypical and neoplastic cells of the gynecologic ract. This work is being integrated with the evaluation of new and experi- ental techniques for specimen collection, processing and staining which are upported by collaborative research contracts from the NCI Committee on ytology Automation. More details about the automation in cytology project s included in Dr. Herman's report.

uring the year Dr. Herman completed and published several studies on epilepsy roducted by focal stimulation of the hippocampus with penicillin (29,30,31 nd 52). This experimental work was performed in collaboration with scientists n NINDS.

ltrastructural Pathology Section:

his was a difficult year for the technical staff in the EM laboratory of this ection because there was no electron microscopist directing the work during he past year following Dr. Grimley's resignation in the Spring of 1973. Miss rene Berezesky resigned in September. Miss Mims has worked with Dr. Braylan n scanning EM of normal and neoplastic hematopoietic cells. Mr. Michelitch as completed the technical aspects of the studies on lymphocytes in L.E. atients started by Dr. Grimley and has also continued to prepare EM sections n current surgical and autopsy sections. Several studies by Dr. Grimley were ompleted and reported during the year. These included EM observations on a yxosarcoma in a rabbit and studies on the morphogenesis of C-type and A-type articles in cells infected with an arbovirus. Also recently published by

Drs. Glenner and Grimley is an AFIP Fascicle on Tumors of the Extra-Adrenal Paraganglion System.

Dr. Henson has been working on the production of hybrid antibodies for the past two years. During the past year he used hybrid antibodies successfully to label herpes virus induced neoantigens on infected cells and in EM photomicrographs could visualize the surface binding sites of the hybrid antibody with ferritin labeling. He used similar hybrid antibody techniques together with perioxidase binding to study the localization by light and EM microscopy of viral antigens in salivary glands of mice chronically infected with CMV. These same reagents and techniques will be used in collaboration with Dr. Triche to study the localization of membrane surface receptors after he starts working in the EM laboratory on July 1, 1974. Dr. Henson also published his study on the neurovirulence of equine herpes virus in mice (26).

Hematopathology Section:

Dr. Berard and his staff have major responsibilities for diagnostic services for patients with diseases of the hematopoietic and lymphoreticular tissues. These include not only the diagnosis of material from Clinical Center patients but also from patients in the cooperative studies of the Eastern Cooperative Oncology Group and cases sent to him in consultation. In fact, there were approximately 450 ECOG and consultation cases which he reviewed and diagnosed during the past year. In addition, he was the author or co-author of a number of papers on studies of malignant lymphomas and participated in several major conferences (International Symposium on Non-Hodgkin's Lymphomas, Tutorial on Neoplastic Hematology, and was co-director of the International Academy of Pathology Long Course on the Reticuloendothelial System).

As noted in last year's report Dr. Jaffe and Dr. Berard had started a major research program in collaboration with Drs. Green, Shevack and Frank (NIAID) to study surface membrane markers of lymphoreticular cells. Dr. Braylan, who joined Dr. Berard's staff as a Senior Staff Fellow in July 1973, and who is studying surface Ig and scanning EM morphology of normal and neoplastic cells participates in this group research project. This research has progressed rapidly and more than 120 specimens from approximately 100 patients have been examined for immunoreceptor sites. One paper (53) about these studies was published in 1973 and 4 papers are currently in press. In brief, these studies have shown that all nodular lymphomas of poorly differentiated or mixed-cell type have the membrane surface markers of "B" lymphocytes and that localization of these neoplastic "B" lymphocytes in the spleen and lymph nodes mimics the localization of normal "B" lymphocytes. Cases of diffuse lymphoma often also have surface receptors of "B" lymphocytes but a few have "T" lymphocyte markers. A few cases of "histiocytic" lymphoma have been studied, particularly cases of histiocytic medullary reticulosis, and the neoplastic cells were demonstrated to have surface receptors for cytophilic antibody characteristic of monocyte-macrophages and lacked the surface markers of "T" and "B" lymphocytes. These results add new and important functional information to the morphologic study and classification of the malignant lymphomas and leukemias. Obviously these same techniques will now be applied to other types of lymphoma and leukemia and to the study of cellular infiltrates and inflammatory reactions to a variety of other neoplasms and non-neoplastic diseases.

Comparative Oncology Section:

Drs. Dawe, Banfield, Snell, Stanton and Dunham are the professional staff members of this Section. Dr. Mulay retired in September 1973 and Dr. Dunham will retire at the end of May 1974.

The experimental studies include: (1) morphologic characterization of neoplasms in a variety of laboratory animals including rodents, primates, lower vertebrates and invertebrates; (2) mechanisms involved in tumor development; (3) chemical and particulate fiber carcinogenesis; (4) cellular interactions between epithelium and mesenchyme; and (5) qualitative and quantitative analysis of elements using SEM and the electron probe. Publications included a study of the ultrastructure and histochemistry of endocrine cells and argyrophil carcinoids of the stomach in Mastomys (Snell 20,41); a review of comparative neoplasia (Dawe 32); and reports and editorials about the carcinogenesis of asbestos, fibre glass and other particulate material (Stanton 37,38 and 39).

Mr. Ralph Isenburg provided photographic services for the experimental studies and photomicrography for publications by the staff of the Laboratory of Pathology.

Viral Oncology and Molecular Pathology Section:

Dr. Friedman spent most of 1973 in England as a visiting scientist at Mill Hill where he worked with a cell free protein synthesizing system to study the mechanism of action of interferon. The major findings were that the antiviral activity of interferon can be assayed in such a system and that the results to date indicate that the action is directed toward the initiation of virus-directed protein synthesis. Dr. Levin has worked primarily with murine oncornaviruses and has demonstrated defective forms of these viruses and characterized their biochemical properties.

Dr. Rabson and his associates have continued their work with herpesviruses and adenovirus-SV40 hybrids. In work with the oncogenic monkey herpesvirus, H. saimiri, they and Dr. R. Dolin of NIAID have shown that this virus provides a helper function for human adenoviruses in monkey cells. In collaboration with Dr. George Klein of the Karolinska Institute and Drs. Gary Pearson and Dharam Ablashi of NCI they have carried out antibody studies with H. saimiri in marmosets and owl monkeys. They have studied antibodies to both early and late antigens and have shown a relationship of antibody to early antigen to tumor induction. The system has remarkable similarities to EBV infection in humans. In collaboration with Dr. A. Lewis of NIAID, morphological characteristics as well as serological and biochemical characteristics of hamster cells transformed by a variety of non-defective adenovirus 2-SV40 hybrid viruses have been described. The morphology of the tumors seems to be dependent on the adenovirus genome rather than the varying amounts of SV40 genetic material in the hybrids. In collaboration with Drs. S. Rosen and B. Weintraub of NIAMDD studies with the ChaGo line have continued. It has been shown that the cells produce more of the alpha subunit of hCG than of the beta. Some clones produce primarily alpha while others produce beta. The cells also produce placental alkaline phosphatase.

Pathological Technology Section:

Miss Sanders and her staff processed and stained approximately 33,000 slides during 1973 for staff in the NCI including staff in the Laboratory of Pathology. In addition approximately 14,000 H&E slides were provided by the contractor. Miss Sanders has now completed plans to move a part of her staff to Frederick, Maryland, where she will operate and develop a histopathology section laboratory in conjunction with staff of the Litton-Bionetics. Plans are for this move to take place in June 1974.

Serial No. NCI 500
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

roject Title: Histogenesis and Pathology of Induced and Spontaneous Tumors of Laboratory Animals

revious Serial Number: Same

rincipal Investigator: Katharine C. Snell, M.D.

ther Investigators: None

ooperating Units: None

an Years (July 1, 1973 - June 30, 1974)

Total:
Professional:
Other:

roject Description:

A. Praomys (Mastomys) natalensis: (Mastomys)

Objectives: (1) To study and describe the tumors and other lesions that evelop spontaneously, some of which appear to be counterparts of human iseases. (2) To study the effect of 2,7-FAA administered orally to Mastomys.

Methods Employed: (1) Mastomys are allowed to live as long as possible o furnish information on tumor incidence. (2) Mastomys were given 2,7-FAA ixed with their food for periods up to about 1 year. Sections from these nimals are being examined.

Major Findings: (1) Malignant argyrophilic carcinoids occur in 1/2 or ore of Mastomys 18 months of age or older. Thymomas and thymic hyperplasia, ometimes associated with myositis and myocarditis, appear in a high perentage of animals. Other relatively common tumors are hepatomas, tumors of he endocrine glands, and of the testis, ovaries or uterus. (2) Mastomys reated with 2,7-FAA developed carcinoma of the liver (100%), some with etastases in various organs, and parenchymatous adenomas of the pancreas 100%). One half of the females developed adenoacanthomas of the mammary land, a tumor never seen in untreated Mastomys. No increase in the incience of gastric carcinoids was observed, probably because few of the treated nimals survived more than 1 year.

Significance: (NCP Objective 3, Approach 1) Mastomys are becoming of increasing importance as experimental animals, since in many ways their diseases parallel those of man. The continued study of untreated Mastomys is important in order to establish a baseline for future experimental work.

Proposed Course: Study of nearly 600 untreated Mastomys has been completed and a paper giving the overall pattern of their neoplasms and other lesions is in the early stages of preparation. A paper is being written concerning the effect of 2,7-FAA.

B. Study of the Offspring of Pregnant Rats Treated with Diethylstilbesterol

Objectives: To see whether it is possible to induce tumors in the offspring of rats by treatment of the mothers when pregnant and thereby shed more light on the recent work of Herbst et al (New England J. Med. 284: No. 16, 1971) and of Greenwald et al (New England J. Med. 285: No. 7, 1971). These authors found that the rare tumor, adenocarcinoma of the vagina, was appearing in a number of young women whose mothers were treated with diethylstilbesterol during pregnancy for threatened abortion.

Methods Employed: Pregnant F344 and SD/N rats were treated by the administration of diethylstilbesterol by stomach tube in doses equivalent for the rat to those given pregnant women. The drug was given on days 12 through 15 of pregnancy. Some of the offspring of these rats were killed when they were about 1 year of age; the rest will be allowed to live out their life span.

Major Findings: Rats killed at 1 year of age showed no significant lesions, hence the lengthening of the period of observation.

Significance: (NCP Objective 3, Approach 1) If tumors are induced in offspring of treated rats, it will help substantiate the inferred relationship in the human patients of prenatal diethylstilbesterol and the development of genital tract tumors.

Proposed Course: Complete the experiment.

C. Untreated Rats

Objectives: To determine the incidence of tumors and other lesions that occur spontaneously in the inbred Sprague-Dawley strain of rats. This is a relatively new strain developed by Dr. Carl Hansen, Laboratory Aids Branch. It is being used by several investigators but no pathologic study has been made on the animals.

Methods Employed: A number of rats were killed at 3-month intervals beginning when the animals were 12 months old. Since these showed no lesions except pneumonia and ear infections, the remainder will be allowed to live out their life span.

Major Findings: None so far.

Significance: (NCP Objective 3, Approach 1) A careful pathologic study will be useful to any investigator using these rats for experimental purposes because it is important to know the spontaneous incidence of tumors and other lesions. Also the knowledge gained from a study of neoplasms and other lesions that occur in laboratory animals can provide a basis and possible analogy to the study of the tumors and other lesions that occur in human beings.

Proposed Course: Complete the experiment.

D. Monkeys

Objectives: Originally the objective of this experiment was to determine whether 3-methylcholanthrene injected into the wall of the stomach would induce gastric carcinoma. Since, after many years of observation, no gastric cancers have been found, we are keeping the remaining monkeys to study any tumors or other lesions that may occur with age.

Methods Employed: 3-methylcholanthrene was injected or implanted in the wall of the stomach of rhesus monkeys 18 to 19 years ago. The monkeys, which are now 24 or more years old are being observed and allowed to live as long as possible. Prior to necropsy, skeletal surveys will be made by Dr. Doppman of the Clinical Center X-ray department. Also, brains will be perfused by Dr. Henryk Wisniewski of the Albert Einstein College of Medicine, who is nterested in studying the aging process of neurons by electron microscopy.

Major Findings: Nothing significant has been observed in the stomach of any monkey necropsied. Three cases of spinal ankylosis, bilateral carcinoma of the kidney, diabetes with hyalinization of the islets of Langerhans, and adenocarcinoma of the ascending colon are the principal pathologic findings so far observed.

Significance: (NCP Objective 3, Approach 1) With the increasing use of monkeys in cancer research it is important to study old animals to determine the incidence of spontaneous disease. Very few colonies of monkeys of known and advanced age are in existence so that our animals constitute an important group for future study. Hyperostotic spondylosis is a disorder that is currently the subject of considerable clinical study. These monkeys may offer an opportunity for observing changes in bone which are almost never available in human autopsy material. The finding of carcinomas of the kidney and of the colon as well as the hyalinized islets in the diabetic monkey uggest that monkeys, if allowed to live long enough, may indeed develop many of the same diseases found in man.

Proposed Course: Continued observation of monkeys with necropsies as necessary with collaborative studies as indicated under Methods.

Publications:

1. Håkanson, R., Larsson, L.I., Owman, C., Snell, K.C., and Sundler, F.: Fluorescence and electron microscopic histochemistry of endocrine-like cells in gastric mucosa and argyrophil tumor of Praomys (Mastomys) natalensis. Histochemie 37: 23-38, 1973.

2. Capella, C., Solcia, E., and Snell, K.C.: Ultrastructural study of the endocrine cells and argyrophil carcinoids of the stomach of Praomys (Mastomys) natalensis. J. Natl. Cancer Inst. 50: 1471-1485, 1973.

3. Stewart, H.L., Dunn, T.B., Snell, K.C., and Deringer, M.K.: Tumors and proliferative lesions of the respiratory apparatus of mice. In Turusov, V.F. (ed.): Pathology of Tumors of Laboratory Animals, Vol. II: Tumors of Mice. Lyon, France, I.A.R.C., in press.

4. Stewart, H.L., and Snell, K.C.: Diseases of Praomys (Mastomys) natalensis resembling those of man. I.L.A.R. News, in press.

5. Stewart, H.L., and Snell, K.C.: Patterns of neoplastic and non-neoplastic diseases of Praomys (Mastomys) natalensis. Recent Results in Cancer Research, in press.

Serial No. NCI 501
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

roject Title: Carcinogenesis in the Cheek Pouch of Hamsters or in the Esophagus of Hamsters: A Search for Factors in the Environment that may be Related to Cancers in Man

revious Serial Number: Same

rincipal Investigator: Lucia J. Dunham, M.D.

ther Investigators:

an Years (July 1, 1973 - June 30, 1974)

Total: 3/4
Professional: 1/4
Other: 1/2

roject Description:

Objectives: To identify substances or factors in the human environment hat induce or influence the development of cancers.

Methods Employed, Major Findings, and Significance: (NCP Objective 1, pproach 1). See the attached Summaries of 2 articles that have been prepared for publication. See also the list of 5 reports on these and other ubjects in geographic pathology of cancer. Two reports have been published, is in press, and 2 are in final draft.

Proposed Course: To submit articles 4 and 5 for publication. To retire rom the Laboratory of Pathology in May 1974.

ublications:

1. Dunham, L.J., Bailar, J.C., III, and Laqueur, G.L.: Report on istologically diagnosed cancers in 693 Indians of the United States, 1950-965. J. Natl. Cancer Inst. 50: 1119-1127, 1973.

2. O'Gara, R.W., Lee, C.W., Morton, J.F., Kapadia, G.J., and Dunham, .J.: Sarcoma induced in rats by extracts of plants and by fractionated xtracts of *Krameria ixina*. J. Natl. Cancer Inst. 52: 445-448, 1974.

3. Dunham, L.J.: Tests of plants from Curacao for carcinogens affecting esophagus: A preliminary report. Presented at International Seminar on the Epidemiology of Esophageal Cancer, Bangalore, India, November 4, 1971. Proceedings, in press, January 1974.

4. Dunham, L.J., Sheets, R.H., and Morton, J.F.: Proliferative lesions in hamster cheek pouch and esophagus treated with plants from Curacao, NA. To be submitted to J. Natl. Cancer Inst., March 1974.

5. Dunham, L.J., and Sheets, P.H.: Effects of esophageal constriction on benzo/a/pyrene carcinogenesis in hamster esophagus and forestomach. To be submitted to J. Natl. Cancer Inst., March 1974.

SUMMARY

Dunham, L.J., Sheets, R.H., and Morton, J.F.: Proliferative lesions in hamster cheek pouch and esophagus treated with plants from Curacao, N.A.

Attempts were made to develop a reliable test for the presence or absence of carcinogens in food and drink. Certain plant materials suspected of affecting the development of esophageal cancer in man were applied to hamsters' upper gastrointestinal tract, including cheek pouch, in several long term experiments. The materials tested, often with added calcium hydroxide, were 9 plants from Curacao used in native teas and remedies, and a tobacco (in snuff), and an alkaloid (arecoline) present in betel quid. Lesions that developed after prolonged treatment with the plants *Annona muricata*, *Heliotropium ternatum*, *Krameria ixina*, and *Acacia villosa*, and with arecoline, were a superficial spreading carcinoma of pouch epithelium, papillomas of esophagus (4), and advanced atypias (1 in pouch and 3 in esophagus). The lesions developed in relation to the cheek pouch route of application only, while none resulted following ingestion of a concentrated tea (*Acacia villosa*), or when administered in the diet (*Annona muricata*, arecoline). The types and distribution of the lesions produced in cheek pouch and esophagus suggest that they were caused by weak carcinogens.

SUMMARY

Dunham, L.J., and Sheets, R.H.: Effects of esophageal constriction on benzo/a/pyrene carcinogenesis in hamster esophagus and forestomach.

In order to constrict the lumen of the hamster esophagus, polyethylene cuffs were applied at operation to a position encircling the esophagus just above its junction with the forestomach. This procedure resulted in such features as atrophy of epithelium at the level of the cuff, and proximal dilatation of the esophagus with hypertrophy of muscle. The carcinogen benzo/a/pyrene (BP) in dimethyl sulfoxide vehicle was instilled twice weekly over a 5 weeks period in the esophagi of hamsters with and without the constricting cuffs, eventually causing papillomatous lesions and atypias in esophagus and papillomas in forestomach of a number of hamsters that survived

o ages over 9 months. Though lesions developed in esophagus and forestomach
n hamsters of both groups treated with BP, there were distinct differences
n the types of lesions and in the ages at survival. In the presence of
onstriction relatively more papillary lesions, one a carcinoma, developed in
he esophagus and at younger ages. In contrast, there were fewer gastric
apillomas per hamster so affected, even though the hamsters were older than
hose with gastric papillomas in the non-constricted group. Gastric papillo-
as that had progressed to cancer were found only in hamsters without con-
triction of the esophagus.

Serial No. NCI 504
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: An Electron Optical Investigation of Normal and Pathologic Tissue

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: G. Kasnic, C.J. Dawe, W. Morgan, C.W. Lee, M. Ohmori

Cooperating Units: Okayama University Medical School, Okayama, Japan

Man Years (July 1, 1973 - June 30, 1974)

Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives: 1. To describe and interpret the ultrastructure of selected pathologic states and to compare the results with suitable controls.

2. To look for and study the inciting agent or accompanying agent in neoplastic and other disease states using human, animal and tissue culture material.

Major Findings: 1. A dense granule, usually single, is found in each mature particle of the pox viruses. At first glance it varies from round to lancet-shaped, width 3.5 to 24 nm, length 10 to 41.5 nm, area 25 to 1800 nm^2. On close examination they are often 5 sided or roughly hexagonal. There are 3 distinct groups according to size based on area in section; Shope fibroma, the largest; rabbit pox; myxoma; cow pox and canary pox in the intermediate group, and vaccinia in the smallest group. The granule is also present in the developmental forms of the virus, outside of disintegrating particles and less commonly in the viroplasm. The granules near disintegrating forms and in the viroplasm are often more than 1.5 times larger than the largest measured in the mature forms.

Significance: (NCP Objective 5, Approach 4) 1. The dense granule may be of fundamental functional importance in the development of the virus and in its effects on the host.

2. This will be the first publication of any atlas on the ultrastructure of the mosquito. See publications.

Proposed Course: 1. A major effort will be made to characterize the ranule chemically by histochemical and other techniques and to isolate it.

blications:

1. Schaff, Z., Grimley, P.M., Michelitch, H.J., and Banfield, W.G.: ontaneous myxosarcoma in a cottontail rabbit (*Sylvilagus floridanus*): servation of tubular structures in the endoplasmic reticulum of tumor cells. *Natl. Cancer Inst.* 51: 293-297, 1973.

2. Ohmori, M., and Banfield, W.G.: The Ultrastructure of the Mosquito, *ëdes aegypti (L)*. Saikon Publishing Co. Ltd., Tokyo, in press.

Serial No. NCI 504b
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: An Investigation of the Collagenous Connective Tissues and Elastin

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: C.K. Lee, L. Sokoloff, C.W. Lee, A. Hough

Cooperating Units: NIAMD, Lab. Exp. Path., Section on Rheumatic Diseases

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular components.

Major Findings: 1. Fibrous long-spacing collagen is reported to occur in human myocardium particularly around the myocardial vessels. Attention is focused on a new type of collagen, "spiney" collagen, that also occurs in the myocardium. Both are abnormally increased in acute rheumatic fever.

2. Identification of the osteochondral junction and tide mark in normal joints and the EDAX (Energy Dispersive Analysis by X-ray) on these structures. The osteochondral junction does not seem to be hypercalcified as reported in the literature. So far no elemental concentration has been detected which might explain the hematoxylinophilia of the tide mark. High concentrations of iron have been found in the chondrocytes of arthritic joints in hemophilia.

Significance: (NCP Objective 5, Approach 2,6) The mucoprotein collagen complex as expressed in the FLS type collagen fibrils may be an integral part of the connective tissue system. These fibrils are present, often in large numbers, in thyroid medullary tumors, Hodgkin's disease lymph nodes and other tumors. Their significance with respect to the metabolic state of the tissue in which they are found should be explored.

Proposed Course: Apply the scanning electron microscope with the energy dispersive x-ray analyzer to the analysis of connective tissues, particularly elastin and elastotic degeneration (senile elastosis).

Serial No. NCI 505
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

roject Title: Evaluation of Potential Pulmonary Carcinogens, Asbestos, Tobacco, Silicates and Heavy Metals and their Relation to the Physiological Status of the Lung

revious Serial Number: Same

rincipal Investigator: Mearl F. Stanton, M.D.

ther Investigators: E. Miller, M. May, and B. Sanders

ooperating Units: Johns-Manville Research and Engineering Center, Manville, New Jersey; Owens-Corning Fiberglass Corp., Toledo, Ohio; Dr. Fred Bock, Roswell Park Memorial Center for Cancer Research; Pharmacopathics Inc., Contract NCI DCBD-N01-CB-33887

an Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/4
Other: 1/3

roject Description:

Objectives: (1) Investigations into pulmonary carcinogenesis with obacco products and other potential pulmonary carcinogens for man. 2) Investigations into the factors affecting development of mesotheliomas xperimentally induced with asbestos, fibrous glass and related fibrous inerals.

Major Findings: The major effort of the year has been to bring together hat data from the series of experiments on intrapleurally implanted fibers f asbestos, glass, and Al_2O_3 initiated 2+ years ago. In preparation is a aper summarizing the findings of 23 experiments on different sized glass ibers. Assessing particle size in these experiments has been particularly edious.

Preliminary results are also in on the intrapulmonary deposits of igarette smoke fractions. It seems clear from these results that the most ctive fraction is the polynuclear aromatic hydrocarbons of the neutral raction; this confirms skin-painting data and indicates a common mechanism.

Examination of potential chemical pulmonary carcinogens is being carried out using the intrapulmonary pellet method. We hope to extend studies on tobacco smoke condensates to more specific fractions and to further examine other potential carcinogens such as extracted products of air-sample collected air-pollutants and specific chemicals such as benzene, carbon tetrachloride and the many specific carcinogenic hydrocarbons found in low levels in cigarette smoke and general atmospheric pollutants.

Secondly a large series of experiments concerning the response of the pleura of the rat to various fibrous carcinogens and related materials have progressed. Preliminary conclusions indicate that fibers greater than 3.0 microns are far less carcinogenic than fibers below this range. Further fibers of varying short lengths i.e., 3-10 microns are far less carcinogenic than longer fibers. These data apply equally to the asbestosis, glass and Al_2O_3 whiskers. These experiments are now taking several courses: A. Establish the specificity of the carcinogenic response; B. Investigate diverse types of fibers (see below); C. Investigate other structural minerals and metals; D. Narrow the dimensional parameters relevant to the carcinogenicity of glass, asbestos and fibrous Al_2O_3. These experiments all require some 2 - 2 1/2 years for completion. During this interim the experiments previously reported on preliminary data will serve as a fruitful source of several publications.

Significance: (NCP Objective 1, Approach 1)

Proposed Course: A large-scale contract, funded by DCCP has been undertaken to test the screening ability of the pulmonary pellet method to detect differences between cigarette smoke condensate from cigarettes with high and low nicotine content. In our own laboratory, we intend to test the method with several innovative cigarette smoke condensates and to explore the system with smoke condensates of other types that are common air pollutants. Briefly our plans call for testing nicotine and various fractions of cigarette smoke condensate and the condensate of cigarette smoke from cigarettes with altered or reduced tar and nicotine content as well as extracts from specific air pollutants and other potential pulmonary carcinogens that may be lipid soluble.

Work with fibers involves the tedious assessment of fiber sizes and separation of fibers into specific size groups for testing utilizing asbestoses, glasses, aluminum oxide, silica, iron and carborundum fibers. The effects of reduction in durability as a result of pyrolysis and the effect on surface properties by fiber coating will be investigated as time and space permit. Electron microscopy of candidate specimens of fibers and fine structure analysis of the early metaplastic alterations in pulmonary epithelium are in progress with the aim of understanding the preneoplastic changes that may be related to the induction of cancer.

In regard to fiber carcinogenesis, asbestos causes cancer not because it is asbestos, but because it contains durable fibers of exceptionally small diameter that are of exceptionally long length. The important corollary to this fact is that any durable fiber of comparable dimension may be as arcinogenic as asbestos. Our current efforts are being directed to searching nd testing many types of fibers that are liable to contact with humans in ne way or another. In particular, aerosols from dust-producing materials anging from fertilizers to pollens as well as large diametered insulation aterials are being examined. Furthermore, fibrous materials ingested orally re being studied. In general, testing consists of intrapleural application s previously described. However, we have recently begun a series of studies n the effect of intravenously administered fibers. This relates directly to he problem of orally ingested fibrous particles since absorption into the lood stream has been demonstrated in the rat.

ublications:

1. Maroudas, N.G., O'Neill, C.H., and Stanton, M.F.: Fibroblast nchorage in carcinogenesis by fibres. Lancet I: No. 7807, 807-809, 1973.

2. Stanton, M.F.: Tumors of the pleura induced with asbestos and ibrous glass. (Letter to the Editor). J. Natl. Cancer Inst. 51: 315-320, 973.

3. Stanton, M.F.: Some etiologic considerations of fibre carcinogenesis. n Wagner, J.C. (ed.): Biological Effects of Asbestos. WHO Monogr., Lyon, 973, pp. 180-183.

4. Stanton, M.F.: Fiber carcinogenesis: Is asbestos the only hazard? . Natl. Cancer Inst. 52: 630-631, 1974.

5. Stanton, M.F., Hirano, T., and Miller, E.: Experimental induction f carcinoma of the lung. I. Quantitative response of the lung to deposits f methylcholanthrene. J. Natl. Cancer Inst., in press.

6. Stanton, M.F., Layard, M.W., and Miller, E.: Studies with fractionted cigarette smoke condensate in beeswax pellets implanted in the lungs of ats. Symposium on Experimental Respiratory Carcinogenesis, Seattle, ashington, June 24, 1974. To be published in the Proceedings.

Serial No. NCI 516c
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Morphologic Studies on Neoplasms and Preneoplastic Conditions in Relation to Pathogenesis, Etiology and Diagnosis

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: E. Miller, M. May, and B. Sanders and staff

Cooperating Units: Richard Adamson, M.D. and staff (LCP)

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/4
Other: 1/3

Project Description:

Objectives: A study and accessioning of cancer in laboratory animals. Collection and development of competence in special areas of cancer morphology. Studies of histopathological and fine structure alterations related to the pathogenesis of disease induced by oncogenic viruses and other carcinogens.

Major Findings: An ongoing program has been the development of special competence in the histopathological and fine structural aspects of tumors of the lung, bone and mammary glands of rodents. Collection and examination of induced and naturally occurring bone tumors has been in progress for more than 5 years and will continue.

The collection and development of competence in lung tumors is essential for the work previously described and additionally serves as an aid to other investigators in the area and to the NCI Registry of Experimental Cancers. A current project on which Ms. May serves is the study of the fine structure of the early squamous metaplasia preceding carcinoma at the site of implanted carcinogen pellets and the comparison of this change with the metaplasia associated with non-specific pulmonary inflammation which does not progress to cancer.

Interest in mammary neoplasia in rats is an offshoot of current long-term pulmonary experiments. Since over 50% of female strain OM rats develop mammary tumors in the second year of life it is necessary to surgically remove these in our experimental groups. As a result we have correlated data on ncidence, growth rate and morphology in several hundred excised mammary neolasms.

Significance: (NCP Objective 1, Approach 2)

Proposed Course: We have through the past 5-6 years acquired an extensive atalog of neoplasms of all types that develop in aged female Osborne-Mendel ats. This data will be collated in an effort to establish normal incidence ates for our colony. In addition, time will be devoted to training in lectron microscopy, studies of neoplasia in lower animals and preparation of monograph on lung tumours in rats.

ublications:

1. Stanton, M.F.: Tumors of bone in pathology of tumors in experimental nimals. WHO Monogr., in press.

2. Stanton, M.F., and Stewart, H.L.: A review of pathology of tumours n laboratory animals. Volume 1: Tumours of the rat, part 1. Environmental esearch and by the Committee on Animal Models and Genetic Stock, in press.

Serial No. NCI 521a
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Investigations of Mechanisms of Tumor Induction by Polyoma Virus in Mice

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D.

Other Investigators: W.G. Banfield

Cooperating Units: Dr. William A. Gibson, Lab. of Oral Medicine, NIDR

Man Years (July 1, 1973 - June 30, 1974)

Total:
Professional: 6/10
Other:

Project Description:

Objectives: To identify the conditions necessary for the neoplastic response of mouse salivary gland epithelium to polyoma virus. Emphasis is on epithelio-mesenchymal interaction during tumorigenesis.

Previous work from this project has shown that:

1) The salivary gland tumors induced by polyoma virus are of epithelial origin. The strongest evidence for this was provided by experiments in which epithelium was consistently traced to be the source of the neoplastic cells by chromosome-tagging of the candidate cell types (Science 171: 394-397, 1971).

2) Salivary epithelium isolated from the mesenchymal cells of salivary gland failed to yield neoplasms when infected with polyoma virus and placed under conditions favorable to neoplastic development *in vivo* or *in vitro* (Int. J. Cancer 1: 419-450, 1966).

In view of these two major findings and ancillary evidence, a concept was developed to the effect that the polyoma-salivary tumor system is a 3-component system comprised of epithelium, mesenchyme, and virus, rather than simply of epithelium (responder) and virus (effector).

The current objective is to determine the mechanism by which the mesenchyme operates as an essential component in this system.

Methods Employed: 1) To investigate the possible requirement of cell-ell contact between epithelium and mesenchyme for tumorigenesis, the response f epithelium to polyoma virus is studied in a trans-filter culture system n which epithelium and mesenchyme are separated from each other by a illipore membrane (0.45 μ porosity, 20-25 μ thickness) that is permeable to acromolecules but not to cells. This is done with a double-compartmented ealed tissue culture chamber devised by Mr. Morgan. Epithelium is placed n one side, and mesenchyme on the other side of a Millipore filter that ivides the chamber into 2 compartments, and both tissues are infected with irus. These trans-filter cultures are maintained for periods up to 1 year nd more. At serial time intervals the Millipore membranes are removed and esults evaluated by 1) light and electron microscopy of the cells on the embranes; 2) observations of the behavior of the recovered cells in Leighton ube cultures; 3) observation of the tumorigenic capacities of the cells fter subcutaneous transfer to newborn mice.

Major Findings: In 22 infected and 23 control trans-filter cultures, orphological transformation was observed in the epithelial component in 5 f the infected groups and none of the controls. Morphological transformation ccurred in the mesenchyme component in 1 infected culture and in none of the ontrols. Epithelial transformation was noted at intervals of 31, 41, 71, 5, and 375 days after initial isolation of the tissues in culture, while esenchyme transformation was noted at 138 d. Three of the transformed epihelial cultures are being maintained in continuous culture for periods up to 8 months, and the transformed mesenchyme has been carried for 21 months. he epithelial cultures are difficult to subculture, while the mesenchyme ine is easily subcultured. The epithelial lines grow in typical epithelial atterns, as closely coherent pavement-type cells associated with an underying basal lamina, displaying desmosomal attachments to one another, and roducing polyoma virus in abundance. Epithelial proliferation takes place imultaneously with lytic response to virus. The mesenchymal line has a haracteristic fibroblastoid appearance; it does not show lytic effects of irus, but has not yet been tested for virus production. One neoplasm resultng from transfer of the mesenchymal line to newborn mice shows an anaplastic ibrosarcomatous histological pattern. Transfer of relatively small numbers f transformed epithelium (still attached to Millipore membrane) to 7 neworn mice failed to yield tumors. Further attempts to produce tumors with ransformed epithelium are planned.

Significance: (NCP Objective 1, Approach 1,3) These experiments, though ot completed and therefore not fully interpretable at this time, will enable s to establish whether or not actual cell contact is essential in the epihelial-mesenchymal interaction required during tumorigenesis in this system. ifferent mechanisms involved during tumorigenesis would be indicated, deending on whether actual cell-cell contact is required. For example, if ell-cell contact were required, cell fusion (epithelial cell with mesenchymal ell) might be the mechanism involved. Results of this experiment to date lready appear to rule out this possibility. On the other hand, if mere roximity of the two cell types is required, the mechanism involved may be ransfer of some macromolecule, such as messenger RNA, or some segment of transcriptional information. Or it might mean that a relationship similar to that which is required for normal salivary gland development is also required for tumor development, in which case the suggested mechanism would be activation of particular gene sites at which the virus acts to transform the epithelial cell. Summarizing, this project seeks to reveal mechanisms of carcinogenesis at the cell level, so that methods to interfere with these mechanisms can be devised.

Proposed Course: The experiment as outlined will be completed, adding other types of controls. Especially needed are further long-term observations of the uninfected controls and of isolated, infected epithelium placed opposite epithelium in the trans-filter configuration. There is some evidence to suggest that, if carried long enough, non-infected salivary epithelium may undergo "spontaneous" transformation. Also necessary is the transplantation of transformed cells to determine a) tumorigenicity and b) whether the (presumptive) resulting tumors are histologically the same as salivary tumors induced in vivo. We have, during the past year, assisted Dr. William Gibson in the use of this tumor system for studies of enzyme changes during tumorigenesis. Dr. Gibson has found some cytochemical changes that we plan to test as phenotypic indicators of transformation in this system.

In view of the improved techniques for organ culture in closed chambers, Mr. Morgan feels it is now feasible to renew efforts to record transformation by time-lapse cinematography. It is planned to do this in single-compartment chambers in which the interaction between recombined salivary epithelium and mesenchyme will be recorded in the presence of polyoma virus and in the absence of the virus (control).

Serial No. NCI 538
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

roject Title: A Study of Phylogenetic Aspects of Neoplasia

revious Serial Number: Same

rincipal Investigator: Clyde J. Dawe, M.D. (Project Officer)

ther Investigators: J.C. Harshbarger, W.G. Banfield, D. Small, and others

ooperating Units: None

an Years (July 1, 1973 - June 30, 1974)

Total:
Professional:
Other:

roject Description:

Objectives: The continuing major objective of this project is to perform omparative studies of neoplasia in invertebrate and lower vertebrate ani-als. Such studies serve two main needs: 1) To gain a comprehensive view f the biology of neoplasia throughout the animal phyla and thus to achieve more detached perspective of the problem of cancer in man; 2) To find and ake use of neoplastic diseases in lower animals in 2 ways: a) by adapting hem to experimental uses in studies of the properties and mechanisms of enesis of cancer; b) by using them as indicators of environmental tumorigenic gents possibly related to the epidemiology of human neoplasia.

Methods Employed: Neoplasms are collected, either in the field or hrough submittal from outside sources, and are studied morphologically and iologically. Light and electron microscopy are used with fixed material. issue culture, transplantation, and induction methods are applied to study henomena such as regeneration, dedifferentiation, autotomy, graft rejection, umor progression, and others. Much of this work is done in conjunction with he Registry of Tumors in Lower Animals at the Smithsonian Institution, for hich the PI serves as Project Officer.

Major Findings and Activities: A. Much of the reporting investigator's ork in this project has been concerned with organizational, advisory, and ducational activities. Among these were:

1. Organization and chairmanship of a symposium on "Environmental arcinogens in Feral Aquatic Animals" at the XIth International Cancer

Congress in Florence, October 20-26, 1974. The proposed program has been approved by the Program Committee of the Congress.

2. Organization and chairmanship of a symposium on Aquatic Animals as Indicators of Environmental Carcinogens, sponsored by the International Union Against Cancer. This meeting will be held at University College, Cork, Ireland, October 16 to 18, 1974.

3. Serving as one of three guest co-editors of a volume to be entitled Neoplasms in Aquatic Animals, in the series on "Progress in Experimental Tumor Research", edited by F. Homburger.

4. Presented paper (with J.C. Harshbarger) entitled Zoo Animals: Their Contribution to Cancer Research, at annual meeting of American Association for Zoos and Parks in Houston, November 1973.

5. To present paper entitled "Phylogeny, Ontogeny, and Oncogeny" at the Argonne National Laboratories symposium on Oncology, November 1974.

6. Since January 1974, have been serving as consultant to Board of Directors, The Upper Bay Survey. This survey is an ecological study supported by the State of Maryland's Department of Natural Resources for the purpose of identifying the impact of chlorinated hydrocarbon contamination on the ecosystems of the Chesapeake Bay.

B. Investigative Work.

1. The study of a chondrosarcoma in a corn snake, reported last year, has not been actively continued this year. The cell culture line derived from the chondrosarcoma is being maintained for possible future use. The observations on the properties of the cell line in culture will be included with a publication to be prepared by Dr. David Small of the Laboratory of Microbiology and Immunology, NIDR.

2. A culture cell line from a nephroblastoma in a rainbow trout has been isolated and maintained for 1 year. The line grows slowly at room temperature and can be subcultured at intervals of 1-2 months. The original spontaneous tumor, like several others we have seen in rainbow trout, was well-differentiated, showing only slightly atypical glomerulus and tubule formation. In culture, cells show epithelial as well as mesenchymal characteristics.

3. Search for further examples of cholangiomas in white suckers (Catastomus commersonii) continues. Another sampling from Deep Creek Lake is in progress as this report is being prepared. Since this tumor has been seen in only one fish since the discovery of 3 in 12 fish examined in 1963, it appears to be a rare neoplasm, and may not be useful as an indicator of an environmental carcinogen.

4. A preliminary histopathologic examination of gonadal tumors in carp has been initiated upon request from Dr. R. Sonstegard, of the University of

uelph, Canada. These tumors have been found at high prevalence rates in arp in certain locations, and are suspected to be associated with industrial ollution.

Significance: (NCP Objective 1, Approach 1,4) The field studies conerned with tumors in carp and white suckers are relevant to cancer prevention, n that they may lead to disclosure of environmental carcinogens. The fact hat these species are both bottom-feeders suggests that presumptive carinogens may be adsorbed to bottom sediments or concentrated in molluscs in he food chain of these fish. Heavy metals and chlorinated hydrocarbon esticides are known to be carcinogenic, and are also found adsorbed to uspended as well as sedimented particulates in aquatic environments.

Proposed Course: The field search for cholangiomas in suckers will be ontinued through the present year. If no further tumors are found, this egment of the project may be terminated.

The collaborative histopathologic study of gonadal tumors in carp will e extended until a satisfactory histogenetic classification can be ccomplished. Further participation in this investigation will depend upon he outcome of this initial work as well as on Dr. Sonstegard's future field tudies of these tumors.

The study of the trout nephroblastoma will be extended to include attempts o transplant the cell line to rainbow trout hosts, intraperitoneally and ntra-ocularly. Induction of tubulogenesis in the cultured cells will be ttempted by using embryonic ventral spinal cord as the inducer. If uccessful, this would demonstrate responsiveness of neoplastic nephrogenic ells, and would suggest that such neoplasms might be subject to a certain mount of artificial control.

ublications:

1. Dawe, C.J.: Comparative neoplasia. In Holland, J. and Frei, E. eds.): Cancer Medicine. Philadelphia, Lea and Febiger, 1973, pp. 193-240.

2. Dawe, C.J.: Discussion of paper by J.C. Harshbarger: Invertebrate nimals - what can they contribute to cancer research? Fed. Proc. 32: 227, 1973.

Serial No. NCI 560
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Electron Probe and Scanning Electron Microscope

Previous Serial Number: Same

Principal Investigators: William G. Banfield, M.D., Stephen Doty, Ph.D.

Other Investigators: K. Heinrich, and staff of the National Bureau of Standards, C.W. Lee, H. Tipton, R.C. Braylan

Cooperating Units: National Bureau of Standards and Biomedical Engineering and Instrumentation Branch, Division of Research Services

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives: Application of electron probe and ion probe to the analysis of biological specimens.

Major Findings: 1. A method for routinely obtaining frozen sections of briefly fixed tissue thin enough for conventional transmission electron microscopy has been developed. Thin sections of fresh frozen tissue can also be obtained.

2. The construction of a light microscope stage for the ETEC scanning electron microscope, primarily for the study of cathode luminescence is almost completed.

Significance: (NCP Objective 5A, 5B, Approach 2,3,6) 1. The preparation of ultrathin frozen sections allows for localizing the electron beam in the electron probe using transmission scanning. It also allows for immunological procedures and enzymatic reactions that cannot be done on fixed tissue. In addition, the ability to prepare frozen sections for conventional transmission electron microscopy opens the way for assessing artefacts introduced by fixation, dehydration and embedding.

2. This microscope will allow for light optical microscopic visual centering of the object of interest under the electron beam and direct visualization of the cathode luminescence.

Proposed Course: 1. In collaboration with the National Bureau of Standards, continue to work out quantitative analytical methods for biology using the electron probe.

2. Apply the scanning electron microscope, with energy dispersive x-ray analytical attachment (EDAX), to the analysis of connective tissues, particularly elastin, and to the analysis of stratified squamous epithelium, squamous carcinomas and basal cell tumors with especial emphasis on sulfur.

3. With Dr. Braylan, study phagocytic activity of mononuclear cell preparations using EDAX to detect the element phagocytized. Correlation of this activity with surface morphology.

4. Complete work begun with Drs. L. Sokoloff and A. Hough on scanning electron microscopy and EDAX analysis of (a) osteochondral junction of normal joints and (b) arthritic joints in hemophilia. See NCI 504b.

5. Use the light microscope stage constructed for the scanning electron microscope in a study of the cathode luminescence of elastic fibers.

6. Construct a light microscope stage for the ETEC scanning electron microscope to be used for energy dispersive x-ray analysis.

Publications:

1. Doty, S.B., Lee, C., and Banfield, W.G.: A method for obtaining ultrathin frozen sections from fresh or glutaraldehyde fixed tissue. Histochemical Journal, in press.

Serial No. NCI 561
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Collaborative Research and Other Activities

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: None

Cooperating Units: JNCI, DCCP, FDA, IARC-WHO

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/2
Other: 0

Project Description: (Other Activities)

Fifty percent of the normal work week is spent as Scientific Editor of JNCI. Additional time in the normal work week is spent in the following activities: 1. Member, Smoking and Health Review Board-OD; 2. Member, Asbestos Protocol Subcommittee of the HEW Committee on Coordinated Toxicological Research and Related Programs; 3. Project Officer, NCI, DCBD-N01-CB-33887, Small Animal Holding Facility; 4. Project Officer, NCI, DCBD-N01-CB-43912, Hematoxylin Substitute Development; 5. Co-Project Officer, NCI, DCBD-N01-CB-33874, Registry, Tumors in Lower Animals; 6. Co-Project Officer, NCI, DCCP-N01-CP-3374, Standardized Lung Pellet Technique; 7. Member, Editorial Board Monograph Series, IARC-WHO, Lyon, France.

Proposed Course: The following continuing activities are anticipated. I am directly involved as project officer to a major contract with Battelle Northwest to examine response to intrapulmonary deposits of high and low nicotine cigarette smoke condensates. This contract is supported by the Smoking and Health Program which currently is in the office of the Director, NCI. I also act as a member of this program's Smoking and Health Review Board on other segments of the program. I also act as project officer on the contract with Louisiana State University to develop substitutes for hematoxylin and the contract with Pharmacopathics, Inc. on the long-term animal holding facility. Finally, I represent the NCI on an Interagency Working Group, headed by FDA, whose mission is to plan and follow through on a program to experimentally investigate the effects of orally ingested asbestos.

Serial No. NCI 584
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Effects of Cyclic AMP on Mouse Salivary Gland Development *In Vitro*

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D.

Other Investigators: W.D. Morgan, G. Johnson, and I.H. Pastan

Cooperating Units: None

Man Years (July 1, 1973 - June 30, 1974)

Total:
Professional:
Other:

Project Description:

Project temporarily discontinued during this fiscal year, while renovations were being made on laboratory. For description, see 1972-1973 report.

Proposed Course: Some additional work on this project will be done to complete data for publication. At that point a decision will be made as to whether the effects of cyclic AMP on this tumor induction system should be investigated further.

Serial No. NCI 591
1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Migration Inhibitory Factor on the Motility of Rabbit Peritoneal Macrophages

Previous Serial Number: None

Principal Investigators: Willie D. Morgan, Dr. Robert J. Kreb, and Mr. Frank G. Malik

Other Investigators: None

Cooperating Units: Division of Bacterial Products, Laboratory of Allergy, Food and Drug Administration

Man Years (July 1, 1973 - June 30, 1974)

Total:
Professional:
Other:

Project Description:

Time-lapse cinematographic studies of the effect of migration inhibitory factor (M.I.F.) on rabbit peritoneal macrophages in vitro were made. Drs. Robert J. Kreb and Frank G. Malik collaborated with us on this project. We divided the work. They made studies of the effect of M.I.F. on rabbit macrophages, using tissue culture techniques and we studied the effect of M.I.F. on these cells, using techniques of time-lapse cinematography. The cinematography was done in this laboratory, while the tissue culture was carried out in Building 29 in the Food and Drug laboratories. Only the cinematography will be described here.

Objectives: Dannenberg, MacKaness, and others had shown that immunologically committed lymphoid cells secrete a migration inhibitory factor (M.I.F.) that affects the movement of macrophages. They showed that cell movement was greatly retarded in the presence of this factor. The object of this project was to make comparative cinematographic records of sensitized rabbit peritoneal macrophages in (a) Eagle's Minimal Essential Medium (M.E.M.) and (b) Eagle's M.E.M. plus M.I.F.

Methods Employed: A modified multi-purpose tissue culture perfusion chamber was used as culture vessel. This chamber is equipped with carbon dioxide permeable fluid and gas exchange tubes, which permit easy access of fluid and gas to the chamber vault. Rabbit peritoneal macrophages were perfused into the chamber through the fluid exchange tube. And when the cells had settled and formed a monolayer the chamber was carefully opened by removing retaining screws and upper coverglass. Wounds were then made in the monolayer of cells on the lower coverglass by scratching the monolayer with a small spatula. After resealing the chamber, Eagle's M.E.M. or M.E.M. + M.I.F. (x300) was perfused into the chamber (M.I.F. was supplied by Dr. John Houck, Children's Hospital, Washington, D.C.). Chamber cultures were incubated at 36°C in an incubator into which carbon dioxide was perfused at a rate of 4 to 5 milliliters per minute. An inverted microscope and camera were focused on macrophages at the edge of a wound and remained in one fixed position throughout the 2 to 6 days of cinematography. Analysis of motion was done with the aid of an Athena Photo Data Analyzer.

Major Findings: Film data show that control rabbit peritoneal macrophages in Eagle's M.E.M. migrated at a rate of approximately 3000 microns per hour and that test cells with M.I.F. (x300) migrated at a rate of approximately 800 microns per hour.

Significance: The above findings are preliminary but suggest that cinematographic records may be helpful in elucidating the phenomenon of inhibition of macrophage motility in the presence of M.I.F.

Proposed Course: A publication is planned for "The Effect of Migration Inhibitory Factor on the Motility of Rabbit Peritoneal Macrophages". But for now, this project is continuing.

Serial No. NCI 852
1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigators:

Cooperating Units:

Man Years (July 1, 1973 - June 30, 1974)

Total:
Professional:
Other:

Project Description:

Objectives: The objectives of this laboratory are to provide complete diagnostic exfoliative and aspirated cytology as well as to collaborate in various clinical research aspects evaluating therapeutic effects and hormonal status of patients when requested. It is also our goal to continually look into improved and/or newer areas of diagnostic techniques.

The specific objectives include:

1. Work to improve methods for cancer diagnosis.
2. Expansion of aspiration cytology applications.
3. To develop cytologic criteria for aspiration cytology.
4. To improve methodology of chromosomal analysis for medical diagnostic purposes.

Methods Employed:

1. Standard Papanicolaou staining procedure is applied to preparations of various kinds of patient material. The specimens are then screened for various cellular changes, benign and malignant.

2. Special staining techniques involving the use of Fuelgen reaction for nuclear sex chromatin study and special technique of millipore filtration are required almost as daily procedures.

3. Cultures of peripheral blood lymphocytes, bone marrow cells, body fluids and tissue biopsies are routine cytogenetic diagnostic techniques.

Serial No. NCI 852

Patient Material:

The figures compiled below are for the last twelve months.

March 1973 - February 1974

Type of Material Examined:	Accessions	Smears
Vaginal and Cervical	1,478	2,624
Other (Ascitic, pleural, gastric, prostatic, urine, bronchial washings, sputa, spinal fluids, sex chromatin, wound washings, breast, oral, aspirations, chromosome analysis, chromosome tissue analysis, and spinal procedures)	4,017	9,353
Total	5,495	11,977

Institutes	Accessions	Smears
NCI	3,480	7,361
NHLI	323	756
NIAMD	547	864
NIAID	211	771
NINDS	163	334
NIMH	82	164
NDI	205	471
NEI	25	116
NICHD	304	733
REF.	118	321
CC.	37	86
Total	5,495	11,977

Major Findings:

Major contributions of the Cytopathology Section:

1. Providing early diagnosis of neoplasms of the cervix, etc. and metastatic lesions of various sites not oftentimes previously diagnosed.
2. Establishing the definitive usefulness of aspiration cytology as instrumental in providing early and rapid diagnosis.
3. Providing chromosomal analysis for clinical studies.
4. Providing chromosomal banding techniques where recognition of individual chromosomes is of great research interest.
5. Providing cytologic methods to the research projects.
6. Cytologic evaluation of therapeutic effects on the urinary tract epithelium.

Significance: (NCP Objective 1, Approach 1,2,3)

1. The diagnostic value of exfoliative cytology in the clinical management of cancer is an accepted fact. Now aspiration cytology technique has proven its usefulness in establishing definitive diagnosis thereby eliminating the more involved procedure and expense in the preparation for an incisional biopsy.

2. The need and demand for cytogenetic study in both service and research fields is increasing.

Proposed Course:

The primary purpose is to provide a complete and prompt cytopathologic service for the entire staff of the clinical center in various research projects and the development of new and improved techniques within the laboratory is our important forward-looking program.

Work in Progress:

1. In collaboration with Dr. Arnold Dellon (Surgery Branch) bronchial washings on patients with oral cancers are being studied blindly to see whether cancer cells will be found in the absence of known lung metastases.

2. In collaboration with Dr. Edward Craykowski of the Dental Institute a study is being done to find the origin of cells that show nuclei changes resembling those described as Anitschkow cells; such cells are found in the oral cavity.

3. Aspiration cytology material is being compiled for the possible preparation of a handbook.

Publications:

1. Chu, E.W., and Hove, R.C.: The clinician and the cytopathologist evaluate fine needle aspiration cytology. Acta Cytol. 17: 413-417, 1973.

2. Aptekar, R.G., Atkinson, J.P., Decker, J.L., Wolff, S.M., and Chu, E.W.: Bladder toxicity with chronic oral cyclophosphamide therapy in nonmalignant disease. Arthritis Rheum. 16: 461-467, 1973.

3. Plata, E.J., Aoki, T., Robertson, D.D., Chu, E.W., and Gerwin, B.I.: An established cultured cell line (HBT-39) from human breast carcinoma. J. Natl. Cancer Inst. 50: 849-862, 1973.

Serial No. NCI 854
1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Studies on Carcinogenesis, Endocrine Factors and Cytogenetic Characters

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigators:

Cooperating Units:

Man Years (July 1, 1973 - June 30, 1974)

Total: 1
Professional: 1
Other: 1

Project Description:

Objectives:

1. To study the effects of Enovid in rodents.
2. To study the effects of Cytoxan in rodents.
3. To study the *in vitro* viral effects on lymphocytes and fibroblasts (in collaboration with Dr. Rabson)
4. Polyploidization of cells by Cytocalacin and its possible relationship in the induction of malignancies.

Methods Employed:

1. Mice (Balb/c) are fed with Enovid pellets. At end of one year's treatment, the cervical lesions will be transplanted to another animal.

2. Mice (Balb/c)
I.V. Cytoxan (0.1 mg/gm body weight)
I.V. Cytoxan (0.2 mg/gm body weight)
Mice will receive I.V. 0.2 mg/gm body weight at monthly intervals.
Daily urine samples will be collected and screened for G-U tract epithelial changes.

3. *In vitro* cells, lymphocytes and other established cell lines were infected with virus (Herpes I and II cytomegalovirus) for different periods of times. The cells were then harvested for chromosomal analysis.

4. Cytocalacin was used to induce polyploid cells; the cells were then harvested at intervals dependent on the growth rate.

Major Findings:

1. All mice that were fed with Enovid pellets developed vaginal cervical lesions suggestive of cancer. Experiments are now underway to reproduce the lesion for transplantation thereby settling the question of true cancerous state. Transplantation of cervical tissues to 2 month-old mice are presently being carried out.

2. From the patient study here at NIH that Cytoxan may produce epithelial changes mimicking cancer but the changes are reversible upon withdrawal of the drug, a result not in full agreement with the published work. Experimental work is needed to assess further the effects of Cytoxan on genito-urinary tract. The results collected up to the present moment are not very fruitful. The animals will be allowed to live out their lives and autopsies will be performed on them.

3. There were several publications on viral-induced chromosomal damages on established fibroblasts. We are carrying out similar experiments to see the viral effects on in vitro human lymphocytes.

4. Cytocalacin is known to induce multinucleation. Our pilot study supports this fact. However, the induced polyploidy does not appear to be establishable as continuous lines except WI-38 where polyploidy maintains its level 4 months after initial induction by Cytocalacin.

Significance: (NCP Objective 1,5B, Approach 1,2,6)

1. Since millions of women are put on Enovid pills, it is paramount to ascertain presence or absence of deleterious effects of contraceptive preparations.

2. Cyclophosphamide is being used for a prolonged period on patients suffering from a variety of diseases. Its safety margin for the maximal therapeutic effects needs to be ascertained.

3. Viral damages to the human lymphocytes carry paramount clinical implications if demonstrated per chromosomal analysis may prove its definite relationship between virus and cancer. The experiments are designed to show any role of individual susceptibility to viral infections.

4. Polyploidy may be the explanation why cells show malignant changes.

Proposed Course:

1. To study any possible cancer-promoting effects of birth control pills.
2. To study effects of chemotherapy on urinary tracts.
3. Continue to study chromosomal characteristics of established breast cell lines with special interest on the recognition of marker chromosomes by "G-bands".
4. Continue to study in vitro viral effects.
5. Continue to study polyploidy of cells.

Publications:

1. Plata, E.J., Aoki, T., Robertson, D.D., Chu, E.W., and Gerwin, B.I.: An established cultured cell line (HBT-39) from human breast carcinoma. J. Natl. Cancer Inst. 50: 849-862, 1973.

Serial No. NCI 517
1. Laboratory of Pathology
2. Pathological Technology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1973 - June 30, 1974

Part A:

Project Title: Pathological Technology Section

Principal Investigator: Miss Betty J. Sanders

Other Investigators:

Cooperating Units:

Man Years (July 1, 1973 - June 30, 1974)
Total: 10
Professional: 2
Other: 8

1. Project Description:

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition developes and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

July 1, 1973 - June 30, 1974

Number of investigators	108
Number of pieces of tissue	31,823
Number of bottles of tissue	7,685
Number of blocks cut	20,953
Number of blocks cut serially	374
Number of frozen blocks cut	139
Number of blocks recut	590
Number of slides stained H & E	26,330
Number of slides stained special	6,238
Number of unstained slides	4,988

Contract Work - Baker Histology Laboratory

Number of slides stained H & E	16,500

2. The Chemical Rubber Company, publisher of a series of standard reference sources, plans one titled, "The Handbook on Laboratory Animal Science." I was invited to write a section on animal histology procedures by the editors, Dr. Melbly and Dr. Altman. A draft was completed and submitted to the editors on November 1, 1973. It is scheduled for publication in 1974.

3. Plans have been submitted to FCRC on renovation of 1320 sq. ft. of space in Building 539. That renovation will be completed by June 1, 1974 for the first phase of Project 14. Five NIH employees of the Pathological Technology Section will transfer to FCRC on that date and prepare to train 5 Litton employees to be hired on July 1, 1974. The Tissue Block Library will also be moved.

   Renovation of an additional 1220 sq. ft. of space should be completed by January 1, 1975.

4. The Baker Histology contract has been extended to June 15, 1974. It is hoped that with additional help from the Litton Bionetic trainees the Baker contract can be cancelled.

Serial No. NCI 853
1. Laboratory of Pathology
2. Surgical Pathology and
   Postmortem Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Surgical Pathology and Postmortem Sections, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The staff of the Surgical Pathology and Postmortem Sections also carries on independent and collaborative research.

Staff:

Dr. Ralph D. Powell, Jr., Head, Surgical Pathology Section
Dr. Louis B. Thomas, Chief, Laboratory of Pathology and Head, Postmortem Section
+Dr. Richard W. Balasa, Resident
+Dr. Peter M. Banks, Resident
+Dr. Chester J. Herman, Resident
+Dr. Michael J. Lotz, Resident
+Dr. Maria M. Tomaszewski, Resident
+Dr. Frank G. Witebsky, Resident
*Dr. William C. Roberts, Chief, Laboratory of Pathology, National Heart and Lung Institute
*Dr. Allen W. Cheever, National Institute of Allergy and Infectious Diseases
*Dr. Aubrey J. Hough, National Institute of Arthritis and Metabolic Diseases
*Dr. Thomas M. Tarpley, Jr., National Institute of Dental Research.
*Dr. Marius Valsamis, Chief, Diagnostic Neuropathology, Bronx Municipal Hospital, and Associate Professor of Pathology (Neuropathology), Albert Einstein College of Medicine.

+ - These physicians are full time Residents in the Laboratory of Pathology.

* - These Associate Pathologists spend part time in the activities of the Surgical Pathology and Postmortem Sections.

Project Description:

Objectives: The objectives of the Surgical Pathology and Postmortem Sections are: (1) to provide diagnostic services in pathologic anatomy; (2) to collaborate with National Institutes of Health investigators in research involving use and study of human material; (3) to conduct a residency training program in pathologic anatomy, and (4) to carry out independent research.

1. The service functions of the Sections during the period April 1973 through March 1974 included:

(a) 297 autopsy examinations. Included in this report is a table which shows the number of autopsies performed for each Institute. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Sections participate.

(b) 6017 specimens were accessioned in the Surgical Pathology Section. A complete listing of these specimens, by Institute, is given in the accompanying table. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissue removed for specific research purposes.

(c) Histological preparation and special procedures:

These functions are carried out under the direction of Miss Mary C. Bowling and a complete listing of specimens processed by Institutes is given in the accompanying table.

(d) Photographic Services of the Department

A photographic record is made of the large amount of the pathological material which is handled and studied in the department. The photography is done by the staff and residents with the technical assistance of Mr. L. Kenzie Edwards who also maintains the equipment and carries out much of the photographic processing. Gross photographs and photomicrographs of the pathologic material are provided to the clinical staff on request, are used extensively for conferences and seminars and many are prepared for publication.

REPORT OF POSTMORTEM PATHOLOGY SECTION April 1, 1973 through March 31, 1974

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMD	NIAID	NINDS	NIMH	NIDR	NEI	NICHD	REF.	TOTAL
Autopsies performed	110	26	8	7	9	--	--	--	1	-	161
Autopsies submitted	31	8	1	--	1	--	--	--	--	95	136
Total autopsies	141	34	9	7	10	--	--	--	1	95	297
Blocks of tissue cut	8321	1004	452	440	442	--	--	--	--	67	10726
Blocks of tissue recut	669	90	59	80	75	--	--	--	--	15	988
Blocks serialled	--	--	--	--	--	--	--	--	--	--	--
Total blocks cut	8990	1094	511	520	517	--	--	--	--	82	11714
Slides made	12401	1314	638	684	781	--	--	--	--	696	16514
Slides H&E stained	9048	1047	488	484	490	--	--	--	13	449	12019
Slides special stained	1948	155	110	162	157	--	--	--	1	151	2684
Total slides stained	10996	1202	598	646	647	--	--	--	14	600	14703
Frozen sections	24	1	--	1	--	--	--	--	--	3	29

Serial No. NCI 853

REPORT OF SURGICAL PATHOLOGY SECTION

April 1, 1973 through March 31, 1974

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMD	NIAID	NINDS	NIMH	NIDR	NEI	NICHD	REF.	RES.	TOTAL
Specimens accessioned	4371	307	171	228	99	5	17	15	32	647	125	6017
Blocks of tissue cut	9686	370	566	428	260	7	23	45	59	592	147	12183
Blocks of tissue recut	540	30	41	72	46	-	4	8	2	31	14	788
Total blocks cut	10226	400	607	500	306	7	27	53	61	623	161	12971
Slides made	33141	1143	2155	2010	805	25	101	163	221	5748	648	46160
Slides stained H&E	20930	569	1080	1068	713	14	63	84	135	3376	150	28182
Slides stained special	5006	198	277	469	199	5	8	28	91	275	103	6659
Total slides stained	25936	767	1357	1537	912	19	71	112	226	3651	253	34841
Frozen sections	352	16	83	3	16	-	2	2	-	-	-	474

Total Surgical and Postmortem Pathology Section Report:

Number of blocks cut----------------	24685
Number of slides made --------------	62674
Number of slides stained H&E --------	40201
Number of slides stained special ----	9343

Listed below is a breakdown of the work performed by Mr. Edwards from April 1973 through March 1974:

Time taking photographs:	455 hours
Time processing:	247 hours

No. of black and white negatives - 4 x 5 inches: 1646

Color transparencies - 4 x 5 inches - gross:	1769
35 mm. transparencies - gross:	6300
Total	8069

Prints contracted out - 4 x 5 inches: 6426

A large proportion of the photomicrography is done under the direction of Mr. Ralph Isenburg who provides professional assistance and facilities for the entire staff of the Laboratory of Pathology.

2. The collaborative activities of the staff and residents in the Surgical Pathology and Postmortem Sections are reflected in the summary of research activities listed below:

(a) Distribution of human organs and tissues

At present there are approximately 50 scientists in the various laboratories at the National Institutes of Health who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

(b) Conferences:

The staff and residents take an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly organized solely or jointly by the department, including a Surgical Pathology Conference, Joint Pathologists Conference, Neuropathology Conference, Lymphoma Staging Conference, Medical Breast Cancer Service Pathology Conference, Lung Cancer Staging Conference, and Gross Autopsy Conference.

A. Dr. Ralph D. Powell, Jr.:

1. A study of the natural history of bronchogenic carcinoma and the effect of chemotherapy and radiotherapy on survival with special emphasis on oat-cell carcinoma. In collaboration with Drs. K.B. Lamoureux and R.E. Johnson, Radiation Branch, NCI.

2. The uterine cervix as the presenting site of metastatic carcinoma. (A report of two cases with review of the literature). In collaboration with Dr. P. Chretien, Surgery Branch, NCI, and Dr. S.H. Bennett, Johns Hopkins, Baltimore, Maryland.

3. Nasal biopsy in disorders of taste and small. (A study of nasal biopsies from approximately 300 patients, including many with disorders of taste and smell). In collaboration with Dr. R.I. Henkin, NHLI.

4. Pulmonary disease occurring in an autopsy population of Cairo, Egypt, UAR. (Lung tissue from a year of consecutive autopsy cases is being reviewed. In collaboration with Dr. A.W. Cheever, NIAID.

5. The response of pulmonary alveolar ducts to bronchoconstrictive agents. (In laboratory animals and in surgically-excised human lung, the effect of bradykinin and allied agents is being studied physiologically and anatomically). In collaboration with Dr. H. H. Newball, NHLI.

6. Ovarian morphology in women with anosmia and hypogonadotrophic hypogonadism. In collaboration with Dr. R.L. Goldenberg, Yale University Medical School, Dr. S.W. Rosen, NIAMD, Dr. J.R. Marshall, formerly NICHD, and Dr. G.T. Ross, NICHD.

7. Histologic alterations in squamous carcinoma of the head and neck region after Methotrexate chemotherapy. In collaboration with Dr. P.B. Chretien and A.S. Ketcham, Surgery Branch, NCI.

8. Testicular steroidogenesis and morphology in zinc deficiency. In collaboration with Drs. N.K. Prakash (formerly NHLI), and R.I. Henkin, NHLI.

9. Pneumocystis Carinii Pneumonia: A light and electron microscopic study of 28 NIH Clinical Center patients. In collaboration with Drs. D.E. Powell, Georgetown University Medical Center, and G.W. Geelhoed, formerly Surgery Branch, NCI.

10. Other Activities:

    (a) Participant, Collaborating Center, International Reference Center for Classification of Uterine and Placental Tumors.

    (b) Member, International Association for the Study of Lung Cancer.

Publications:

1. Cook, A.J., Weinstein, M., and Powell, R.D., Jr.: Diffuse amyloidosis of the tracheobronchial tree. Radiology 107: 303-304, 1973.

2. Rosenberg, E., and Powell, R.D., Jr.: Active tumor immunotherapy with BCG. Southern Med. J. 66: 1359-1363, 1973.

3. Sato, N., Haller, E.W., Powell, R., and Henkin, R.I.: Sexual maturation in bulbectomized female rats. J. Reprod. Fertil. 36: 301-309, 1974.

4. Powell, R., Larson, A.L., and Henkin, R.I.: Nasal mucous membrane biopsy in Sjogren's syndrome: A new technique for evaluation of the histologic features. Ann. Intern. Med. In press.

5. Jones, A.E., Larson, A.L., Powell, R.D., Johnston, G.S., and Henkin, R.I.: Localization of ^{99m} Technetium in the region of the nose in Sjogren's Syndrome. Ann. Otology, Rhinology and Laryngology. In press.

B. Dr. Peter M. Banks:

1. Case Report: Histiocytic medullary reticulosis. In collaboration with Drs. D.L. Hurley and S.M. Wolff, NIAID.

2. Case Report: Spontaneous long-term remission of widespread metastatic malignant melanoma; evidence of immunologic mechanisms. In collaboration with Drs. G.B. Bulkley and M.H. Cohen, Surgery Branch, NCI.

3. Review of histiocytic lymphoma with regard to central nervous system involvement. In collaboration with Drs. P.A. Bunn and P.S. Schein, Medicine Branch, NCI.

4. Case Report: Small cell variant of Sezary's syndrome with eventuation as visceral involvement by mycosis fungoides type lymphoma. In collaboration with Dr. Marvin Lutzner and Richard Edelson, Dermatology Branch, NCI.

5. A review of pathologic findings among 28 cases of American Burkitt's lymphoma. In collaboration with Drs. G. Canellos, V.T. DeVita, Medicine Branch, NCI, J. Arseneau (formerly Medicine Branch, NCI), H. Gralnick, Clinical Pathology, CC, and C.W. Berard, Laboratory of Pathology, NCI.

C. Dr. Chester J. Herman:

Since research projects are associated with cytopathology, they are listed under Serial No. 589, Cytopathology Service.

D. Dr. Michael J. Lotz:

1. Pyelitis cystica hemorrhagica. In collaboration with Dr. R. Saldino (formerly DR, CC).

2. Multiple neoplasms in two siblings with a variant form of Fanconi's anemia. In collaboration with Drs. G.P. Sarna, NCI; P. Tomasulo, (formerly NCI); J. Bubinak, NCI, and N.R. Shulman, NIAMD

3. Pathological staging of 100 non-selected non-Hodgkin's lymphoma patients. In collaboration with Drs. C.W. Berard, V.T. DeVita, and R. Johnson, NCI.

Publications:

1. Lewis, R., and Lotz, M.J.: Medullary extension of osteosarcoma. Cancer:33: 371-375, 1974.

2. Lewis, R., and Lotz, M.J.: Estra-osseous osteosarcoma. Am. Surg. In press.

E. Dr. Maria M. Tomaszewski:

1. The incidence of benign and malignant tumors other than breast in patients with breast cancer. In collaboration with Dr. D.E. Henson, LP, NCI.

2. Histopathologic evaluation of morphology of breast cancer in patients 35 years of age and less and 65 years or older. In collaboration with Dr. D.E. Henson, LP, NCI.

F. Dr. Frank G. Witebsky:

1. Review of pathology of Wiskott-Aldrich syndrome cases in our files. In collaboration with Drs. C.W. Berard and Michael Blaese, NCI.

2. The effect of sensory nerve transection on sensory end organs in the skin. In collaboration with Dr. A.L. Dellon, Surgery Branch, NCI.

3. Report of case of dissecting aneurysm of hepatic artery. In collaboration with Dr. H. Hoover, Surgery Branch, NCI.

4. Case report regarding seminal vesicle tumor. In collaboration with Dr. N. Javadpour, Surgery Branch, NCI.

G. Miss Mary C. Bowling:

Publication:

1. Bowling, M.C., Smith, I.M., and Wescott, S.L.: A rapid staining procedure for Pneumocystis carinii. Am. J. Med. Technol. 39: 267-268, 1973.

Serial No. NCI 582
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Pathogenesis of Chronic Mouse Cytomegalovirus Infection in Lymphoid Organs and Effect of Chronic Infection on Development of Lymphoma

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Margery Sullivan; and Duran Harris

Other Investigators: A.J. Strano

Cooperating Units: Armed Forces Institute of Pathology

Man Years (July 1, 1973 - June 30, 1974)

Total: 1
Professional:
Other:

Project Description:

Objectives: Study the mechanisms of latent murine cytomegalovirus infection in the salivary glands and lymphoid tissue of mice chronically infected with this virus and determine effect of chronic infection on lymphoma development.

Methods Employed: Tissue culture, immunofluorescence, electron microscopy.

Significance: (NCP Objective 1, Approach 4) The mouse cytomegalovirus causes chronic infection in the submaxillary glands, lymph nodes, and spleens of many strains of mice. Infection in the lymph nodes and spleen probably occurs in lymphocytes. The murine cytomegalovirus belongs to the herpesvirus group of animal viruses, along with the Epstein-Barr virus and *Herpesvirus saimiri*.

Proposed Course: Investigate 1) latent cytomegalovirus infection in lymphoid tissue and 2) effect of chronic infection on chemical or naturally induced lymphomas in mice. Results to date indicate that chronic infection of mice with mouse cytomegalovirus does not accelerate natural development of lymphoma in AKR mice.

Serial No. NCI 583
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunological, Biological, and Ultrastructural Studies of Leucocytes and Other Cells Infected with Herpes-type Viruses

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Ralph Helmsen, Ph.D.; Margery Sullivan; and Duran Harris

Other Investigators: None

Cooperating Units: National Eye Institute

Man Years (July 1, 1973 - June 30, 1974)

Total: 1
Professional:
Other:

Project Description:

Objectives: 1) Develop immunologic and ultrastructural techniques for examination of surface antigens and receptor sites on human leucocytes and on tumor cells. 2) Study effects of overt and covert infections of herpes-type viruses on distribution and types of surface receptors on leucocytes and other cells.

Major Findings: Hybrid antibody labeling technique has been developed to localize herpesvirus antigen in cultured cells.

Significance: (NCP Objective 3, Approach 5) Much evidence indicates that the lymphoid system is a target organ for herpes-type viruses. Development of immunologic and ultrastructural techniques will aid in our understanding of virus expression in leucocytes in clinical conditions such as lymphomas or lupus erythematosis.

Proposed Course: Hybrid antibody method of immunolabeling for electron microscopy has been developed in our laboratory. System will be applied to tumor antigens and to virus infected cells.

Serial No. NCI 585
1. Laboratory of Pathology
2. Ultrastructural Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Histological Evolution of *In Situ* Carcinoma in the Vagina

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; and Glenn W. Geelhoed, M.D.

Other Investigators: None

Cooperating Units: Surgery Branch, NCI

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/4
Professional: 1/4
Other:

Project Description:

Objectives: Clinical-pathological study of women with multicentric squamous cell cancers in the ectogenital epithelium.

Major Findings: Histological studies made on women with sequential vaginal biopsies have demonstrated progression from severe epithelial atypia, through dysplasia, to carcinoma *in situ*. Like carcinoma of the cervix, *in situ* vaginal tumors have an invasive potential. *In situ* vaginal tumors associated with carcinoma of the cervix seem to form a destructive clinical syndrome.

Significance: (NCP Objective 5, Approach 6) Many women with cancer of the uterine cervix subsequently develop epithelial cancers in the vagina and/or vulva. These multiple cancers have been considered to arise in an epithelial region predisposed to malignancy. This region has a common cloacal origin embryologically.

Proposed Course: Project is still in effect. Patients will be added to the study as they are diagnosed. A report has been prepared for publication.

Serial No. NCI 590
1. Laboratory of Pathology
2. Ultrastructural Pathology
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Clinical Histopathological Studies of Breast Cancer

Previous Serial Number: None

Principal Investigators: Donald E. Henson, M.D.; and Maria Tomaszewski, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1973 - June 30, 1974)

Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objectives: These are twofold: 1) Attempt to relate histopathological findings on breast cancer with prognosis, efficacy of therapy, and other diseases, and 2) Attempt to define new classifications of breast cancer.

Major Findings: Results indicate that patients with breast cancer have a different spectrum of second benign and malignant tumors than female patients with lymphoma.

Significance: (NCP Objective 5B, Approach 4) Breast cancer is a leading cause of death in women in the United States. The significance of these studies is to use histopathological data to guide therapy and predict prognosis.

Proposed Course: Study and classify breast cancer cases at NIH and search for common denominators.

Serial No. NCI 531
1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In Vitro Studies of Human Malignant Tumors

Previous Serial Number: Same

Principal Investigator: Alan S. Rabson, M.D.

Other Investigators: J. Costa, C. Kim, G.T. O'Conor, E.W. Chu, D. Char, M. Blaese, D. Albert (Yale Univ.), S. Rosen (NIAMDD), and K. Takemoto (NIAID)

Cooperating Units: None

Man Years (July 1, 1973 - June 30, 1974)

Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives: To study biological properties of human lymphoma, sarcoma, carcinoma and retinoblastoma cells in vitro and to attempt to isolate oncogenic viruses from these cells.

Methods Employed: Standard tissue culture, virologic and pathologic techniques are used.

Major Findings: 1. A human papovavirus has been isolated from a brain tumor (reticulum cell sarcoma of brain) which arose in a patient with Wiscott-Aldrich syndrome. The child was a patient studied by Dr. Blaese of the Metabolism Branch, and in collaboration with K. Takemoto of NIAID we have succeeded in isolating a human papovavirus from the tumor by cocultivation of explants of tumor tissue with susceptible indicator cell lines. The virus is in the same family as the oncogenic mouse polyoma virus and the oncogenic Simian Virus 40 and studies are now in progress to determine whether the virus is etiologically related to the tumor.

2. Studies have continued with the human lung cancer cell line ChaGo which produces chorionic gonadotropin in vitro. We have been deriving additional clonal lines which have not yet been characterized. In collaboration with Dr. H. Sussman of Stanford University we have shown that the cell line also produces human placental alkaline phosphatase in vitro and that

this enzyme has physicochemical properties similar to the placental alkaline phosphatase isolated from normal human placentas. Attempts to demonstrate viruses in the lung cancer cells with a variety of inducing agents have thus far been unsuccessful.

3. We have continued our studies of retinoblastomas in vitro in collaboration with Dr. D. Albert (Yale University). Some suggestive virus-like particles have been seen in some of the tumor cells by electron microscopy and an enzyme with properties similar to oncornavirus reverse transcriptase has been found in some of the cultures. In collaboration with Dr. Devron Char, we have shown cell mediated immunity against the retinoblastoma cells in culture by lymphocytes from patients with pigmentary retinal degenerations and with retinoblastoma.

Significance: (NCP Objective 3, Approach 2) 1. If it can be shown that the human papovavirus we have isolated from the reticulum cell sarcoma of the brain of the Wiscott-Aldrich patient is etiologically related to the tumor, it will be the first example of a human malignant tumor produced by a virus!

2. Studies of ectopic hormone production by the human lung cancer cell line will give us new insight into mechanisms and significance of ectopic hormone production by human cancer cells and may give information of the nature of derepression associated with malignant transformation.

3. The possibility that human retinoblastomas are induced by viruses remains a reasonable hypothesis and the evidence of cell mediated immunity against these tumors supports this. The demonstration of the cell mediated immunity also suggests that methods to increase this immunity might be of value in therapy.

Proposed Course: Attempts to characterize the human papovavirus isolated from the brain tumor and to establish its role in the etiology of the tumor will continue. Studies of the factors involved in ectopic gonadotropin secretion by lung cancers will continue. Studies of the possible viral etiology of human retinoblastoma will also continue.

Publications:

1. Rabson, A.S., Rosen, S.W., Tashjian, A.H., Jr., and Weintraub, B.D.: Production of human chorionic gonadotropin in vitro by a cell line derived from a carcinoma of the lung. J. Natl. Cancer Inst. 50: 669-674, 1973.

2. Tashjian, A.H., Jr., Weintraub, B.D., Barowsky, N.J., Rabson, A.S., and Rosen, S.W.: Subunits of human chorionic gonadotropin: Unbalanced synthesis and secretion by clonal cell strains derived from a bronchogenic carcinoma. Proc. Natl. Acad. Sci. USA 70: 1419-1422, 1973.

3. Ludueña, M.A., Sussman, H.H., and Rabson, A.S.: Synthesis of human placental alkaline phosphatase in vitro by the ChaGo cell line. J. Natl. Cancer Inst., in press.

4. Char, D.H., Bergsma, D., Rabson, A.S., Albert, D.M., and Herberman, R.B.: Cell mediated immunity to retinal antigens in patients with pigmentary retinal degenerations. Invest. Ophthalmol., in press.

5. Char, D.H., Ellsworth, R., Rabson, A.S., Albert, D.M., and Herberman, R.B.: Cell mediated immunity to a retinoblastoma tissue culture line in patients with retinoblastoma. Invest. Ophthalmol., in press.

Serial No. NCI 332
1. Laboratory of Pathology
2. Viral Oncology and Molecular
   Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Oncogenic DNA Viruses

Previous Serial Number: Same

Principal Investigator: Alan S. Rabson, M.D.

Other Investigators: J. Costa, C. Kim, N. Muñoz, E. Chu, G.T. O'Conor,
P. Perlman, D. Ablashi, and F. Bastian

Cooperating Units: None

Man Years (July 1, 1973 - June 30, 1974)

Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives: To study some biological properties of DNA viruses with proven or possible oncogenic activity.

Methods Employed: Standard virologic and pathologic methods have been used.

Major Findings: 1. Herpesvirus saimiri: Studies of the MLC cell line of marmoset lymphoid cells derived from an H. saimiri induced malignant lymphoma have continued. The cells have been shown to contain the H. saimiri genome by DNA-DNA hybridization and early antigens of H. saimiri can be induced in the cells by treatment with halogenated pyrimidines. In collaboration with Dr. D. Ablashi, it has been found that the cells also contain a soluble antigen of H. saimiri which may be similar to the soluble antigen of EBV. In collaboration with Dr. W. Levinson (Univ. California, San Francisco) we have shown that H. saimiri can be inactivated by the antiviral thiosemicarbazones.

2. H. varicellae: The varicella-zoster virus has been isolated from a spinal ganglion of a clinical center patient with malignant lymphoma and zoster. This is the first time the virus has actually been isolated in culture from such a ganglion.

3. Adenovirus-SV40: In collaboration with Dr. A. Lewis, NIAID, we have completed a histologic study of tumors produced by cells transformed by non-defective hybrids of Adenovirus 2 and SV40. The presence of varying amounts of SV40 genetic material in the hybrids had no effect of the dominant effect of the adenovirus genome of the tumors produced.

4. Cytomegaloviruses and Herpesvirus hominis: We have been continuing studies of human cytomegalovirus and H. hominis type 2 with regard to their relationship to human tumors but thus far we have no evidence for presence of viral antigens in any human tumors or transformation of human cells by these viruses.

Significance: (NCP Objective 3, Approach 2) The mechanisms of repression and possible integration of herpesviruses in mammalian cells and mechanisms of neoplastic transformation may be of extreme importance in understanding the role of herpesviruses in human cancer. Studies with a model system involving a primate oncogenic herpesvirus such as H. saimiri and studies of herpesviruses in human lymphoma cells may be of value in this regard.

Proposed Course: We intend to continue studies of the cell virus relationship in the marmoset lymphoma cells and to examine further the mechanisms of latency with H. hominis and cytomegaloviruses. We plan to prepare mutants of H. hominis type 2 to use to determine the role of this virus in human cervical cancer.

Publications:

1. Klein, G., Pearson, G., Rabson, A.S., Ablashi, D.V., Falk, L., Wolfe, L., Deinhardt, F., and Rabin, H.: Antibody reactions to Herpesvirus saimiri (HSV)-induced early and late antigens (EA and LA) in HSV-infected squirrel, marmoset and owl monkeys. Int. J. Cancer 12: 270-289, 1973.

2. Dolin, R., and Rabson, A.S.: Herpesvirus saimiri: Enhancement of adenovirus-associated virus. J. Natl. Cancer Inst. 50: 205-208, 1973.

3. Paulson, D.F., Fraley, E.E., DeKernion, J.B., and Rabson, A.S.: Virus-induced genitourinary malignancy: Macromolecular identify. Invest. Urol. 10: 298-304, 1973.

4. Lewis, A.M., Jr., Rabson, A.S., and Levine, A.S.: Studies of non-defective Adenovirus2-Simian Virus 40 hybrid viruses. X. Transformation of hamster kidney cells by Adenovirus 2 and the nondefective hybrid viruses. J. Virol., in press.

5. Levinson, W., Coleman, V., Woodson, B., Rabson, A., Lanier, J., Whitcher, J., and Dawson, C.: Inactivation of herpes simplex virus by thiosemicarbazones and certain catiuns. Antimicrob. Agents Chemother., in press.

6. Bastian, F.O., Rabson, A.S., Yee, C.L., and Tralka, T.S.: Herpesvirus varicellae: Isolation from human dorsal root ganglia. Arch. Pathol., in press.

SUMMARY

Tumor Immunology Contract Program
July 1, 1973 through June 30, 1974

An Immunology Program Plan has been designed which has as its goals 1) the use of immunologic techniques to detect agents etiologic for cancer, 2) the prevention of cancer by immunologic manipulations, 3) the use of immunologic techniques to improve the diagnosis of cancer, and 4) to manipulate the immune system of patients with established cancer so that increased survival is achieved.

The Immunology Contract Program has been designed to achieve these goals through the support of investigations relevant to the Plan. Thus far, implementation has been directed toward 1) studies of fundamental problems (designated immunobiology), the solutions to which are required for achieving the four goals, 2) immunodiagnostics, and 3) immunotherapy. All projects have been assigned to one of these three categories although many projects include research in more than one category.

Most of the contracts in the Program are relatively new, and this summary is therefore a statement of areas of research being supported, rather than a summary of the findings within these areas.

A. IMMUNOBIOLOGY

1. Identification and characterization of surface membrane components of tumor cells and cells of the immune system. Projects in this area include studies of (1) tumor associated antigens and corresponding antibodies in human (CB-43921) and animal (CB-02320) systems; (2) biochemical, serologic and immunogenetic characterization of normal and tumor cell surface antigens (CB-43920, CB-43925, CB-43986, CB-43985, CB-43894, CB-43892); (3) organization and dynamics of cell surface membrane components relevant to tumor immunology (CB-33879, CB-43922, CB-43987, CB-43880, CB-44000).

2. Investigations of immunologic reactivity of hosts to tumors and identification of immune cells involved. Projects in this area include (1) studies of differentiation of immune cells and determination of roles of different cell populations in immunologic reactions to tumors (CB-23889, CB-33859, CB-33866, CB-33868, CB-33870, CB-23890, CB-43927, CB-43929, CB-43971, CB-43970, CB-43994, CB-43998, CB-43997); (2) studies of immunoglobulin coating on tumor cells (CB-43858); (3) studies of antigen binding receptors on T cells and transplantable T cell tumors (CB-43923, CB-43999, CB-43924); (4) studies of factors controlling differentiation of tumor cells (CB-23890); (5) studies of mechanisms for tumor cell destruction and means of escape from these (CB-02207, CB-23878, CB-43930, CB-43931, CB-44001, CB-43995); (6) studies of genetic control of immune responses in relation to cancer (CB-43934, CB-43972).

3. Development, maintenance and distribution of special experimental

animals, tumors, and cell lines, and development of improved techniques needed in tumor immunology. Projects in this area include (1) maintenance and distribution of mouse tumors and cell lines (CB-23886 and CB-92142); (2) development, testing and distribution of H-2 recombinant strains of mice (CB-43941, CB-43935, CB-43988, CB-43936); (3) development of improved techniques for *in vitro* sensitization of human lymphocytes (CB-43884); (4) development of reagents for identification of human T and B cells (CB-23882); (5) studies of serum factors affecting *in vitro* cell growth (CB-23883).

B. IMMUNODIAGNOSIS

1. Evaluate use of carcinoembryonic and other fetal antigens in the diagnosis of human cancer. Projects in this area include (1) development of more sensitive assays (72-3890-2); (2) attempts to better understand the chemical composition and structurally characterize fetal antigens (72-3877, CB-33869, CB-43886, CB-43989); and (3) clinical studies of usefulness in diagnosis or monitoring of patients with malignancy (CB-33858, CB-33875, CB-43887, CB-43888).

2. Search for new tumor associated antigens in human cancer which may be useful in immunodiagnosis. Projects in this area include (1) animal models for circulating tumor associated antigens (CB-43891); (2) investigation of other extraction procedures for glycoproteins from gastrointestinal cancer (CB-43859); (3) extraction of antigens and immunization of heterologous species to develop new potentially useful diagnostic reagents (CB-43860, CB-43889, CB-43858, CB-43890).

3. Evaluate diagnostic usefulness of serologic assays and of humoral immune response to tumor associated antigens. Projects in this area include (1) develop animal models and more sensitive techniques for early detection of antibodies to tumor associated antigens (CB-43882); (2) identification of antibodies to autologous human cancer (CB-43881, CB-43953); and (3) serum collections for evaluation of serum diagnostic tests (72-3879, CB-33914, CB-43861, CB-33915).

4. Evaluate diagnostic usefulness of cell-mediated immune response to tumor associated antigens. Projects in this area include (1) improved methodology of assays for cell-mediated immunity (CB-43965, CB-43893); (2) improved procedures for preparation of effector lymphocytes and target cells (CB-43854, CB-43964, CB-43885, CB-43883); (3) isolation of antigens active in in vivo and in vitro assays of cell-mediated immunity (G-69-2176); and (4) evaluate usefulness of assays for depression in cell-mediated immunity in cancer patients (CB-43853).

C. IMMUNOTHERAPY

1. Pre-Clinical Research. Projects in this area include (1) evaluation of neuraminidase (VCN) treatment of tumor cells for vaccine production, in which

dose, route and schedule of administration, evaluation of this modality in metastatic disease, a search for new animal models, and the utilization of VCN vaccine in combination therapeutic approaches are being studied (43864, 33860, and 43865); (2) investigations of new animal models and parameters of application of immunotherapy utilizing BCG or its subcellular components (33891, 33896, and 02208); (3) a search for new approaches to immunotherapy such as the use of anti-B cell serum (92061), evaluation of graft-vs-host (GVH) and rescue from GVH (33853), immunological investigation of the newly-observed effects of amphotericin-B (43872), and development of primate models for bone marrow transplantation (02103); (4) studies on utilization of nitrogen mustard as a means of altering tumor antigenicity and evaluation of hybridization of tumor cells to non-malignant cells as a mechanism for increasing immunogenicity of the tumor cell antigens (RFP 74-6); and (5) studies of transfer factor and immune RNA in animal systems (RFP 74-8).

2. Immunologic Status of Recipients of Immunotherapy. Included in this category are studies of (1) macrophages (43873); (2) B and T cells (43875); (3) in vitro assays of cell-mediated immunity and preparation of human tumor-specific antigens for use in such assays (23227); (4) analysis of the effects of various immuno-potentiators on parameters of the immune response in pre-clinical animal systems (43877); and (5) analysis of the role that freely-circulating tumor antigens may play in response to immunotherapy (43874).

3. Human Studies. Clinical trials of immunotherapy utilizing a number of approaches have been initiated. Projects in this area include studies of (1) BCG, either alone or in combination with allogeneic tumor cells, in the treatment of melanoma, lung and breast cancer and leukemia (43852, 33888, and 43857); (2) transfer factor therapy of sarcoma (43878); (3) neuraminidase treated tumor cells in the treatment of advanced cancer (23885) and melanoma (43852); (4) neuraminidase treated allogeneic tumor cells and MER in acute myelogenous leukemia (RFP 74-9); (5) the effect of "deblocking" plasma on the immune system of patients with melanoma (23887); and (6) topical application of immunotherapeutic agents (33373).

CONTRACT RESEARCH SUMMARY

Title: Development of Antisera Specific for Human T and B Lymphocytes

Principal Investigator: Alexander R. Lawton, M.D.
Name/Address
University of Alabama
The Medical Center
Performing Organization: Birmingham, Alabama 35233

Contract Number: N01-CB-23882
Starting Date: June 20, 1972 Expiration Date: Sept. 1974
Goal: To develop two reagent antisera, one specifically capable of recognizing B lymphocytes and the other capable of reacting specifically with T lymphocytes.

Approach: The contractor will develop these antisera by immunizing rabbits and goats with presumed T lymphocytes obtained from patients X-linked agammaglobulinemia as well as fetal thymus cells. Animals will be immunized with B lymphocytes obtained from patients selectively deficient in T lymphocytes, such as patients with chronic lyphocytic leukemia. Antisera will be appropriately absorbed and specificity tested by cytotoxic and binding activities for appropriate lymphoid cells.

Progress: Several rabbit antisera to human T lymphocytes from fetal thymus or peripheral leukocytes of an agammaglobulinemic donor have been prepared and extensively absorbed. Similar antisera have been raised against lymphocytes rich in B cells, but these are technical difficulties in the absorption of this material. Studies in patients with immunodeficiency and lymphoproliferative diseases are underway.

Significance for Cancer Research (NCP Objective 2 Approach 1)
Manipulation of T and B lymphocytes is a crucial aspect of developing reliable methods of altering immunologic capacity of individuals.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: N/A
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $39,192

CONTRACT RESEARCH SUMMARY

Title: Analysis of Serum Requirements for In Vitro Immunological Studies

Principal Investigator: Robert I. Mishell, M.D.
Name/Address University of California, Berkeley
Berkeley, California 94720

Performing
Organization:

Contract Number: N01-CB-23883
Starting Date: June 28, 1972 Expiration Date: August 1974
Goal: To define serum requirements for immunological studies employing tissue culture techniques.

Approach: Using the mouse spleen in vitro immune response as an assay, sera obtained from several species will be examined for their capacity to substitute for fetal bovine sera. The latter are in very limited supply. In addition, attempts will be made to explore the use of bacterial endotoxin and other agents for their capacity to restore batches of sera that are not useful in the in vitro assays. Furthermore, preliminary fractionation and characterization of both toxic and growth promoting substances will be carried out.

Progress: Sixty-seven lots of fetal bovine, calf, adult bovine, mouse, lamb, and swine sera have been tested for effects on both primary and secondary response in vitro, crude endotoxin and Concanavalin A have been tested and found to restore 16 lots of deficient fetal bovine sera. Complementation tests have been performed on 13 lots of sera, and an initial fractionation has been performed to isolate the supportive activity of positive sera.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Most in vitro studies of cellular immune processes depend on added serum. It is essential that sources of such sera be standardized in order to properly evaluate cancer patients' responses to therapy.
Project Officer: Barbara H. Sanford, Ph,D.
Program: Immunobiology Site Visit Date: April 25, 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $77,316

CONTRACT RESEARCH SUMMARY

Title: Isolation, Characterization and Immunochemistry of Tumor Angiogenesis Factor (TAF)

Principal Investigator: Bert L. Valle
Name/Address
Harvard Medical School
Boston, Massachusetts

Performing
Organization:

Contract Number: NO1-CB-43942
Starting Date: In negotiation Expiration Date:
Goal: To purify and characterize TAF and produce the corresponding antibody.

Approach: TAF will be purified to homogeneity and characterized as to chemical composition. Throughout purification in vivo bioassays will be carried out to confirm its specific activity, i.e. ability to induce neo-vascularization.

Progress: New contract

Significance for Cancer Research (NCP Objective 4 Approach 2)
If neo-vascularization could be prevented by antibody against TAF, tumor growth might be prevented.

Project Officer: Barbara Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Oct. 5, 1973
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $175,000 (Est.)

CONTRACT RESEARCH SUMMARY

Title: The Role of T-lymphocytes in Tumor-Associated Immunity

Principal Investigator: Dr. Birger Andersson
Name/Address Karolinska Institute
S-104 01, Stockholm 60, Sweden

Performing
Organization:

Contract Number: N01-CB-33866
Starting Date: June 25, 1973 Expiration Date: March 1974

Goal: To evaluate the role of B cell immunity in in vivo tumor rejection using specially prepared animals.

Approach: Tumor rejection capacity will be evaluated by inoculation of titrated tumor doses and measurement of antibody production. At least three well-defined tumors will be used in these assays.

Evaluation of T cell effects will be made utilizing at least two different methods of T cell reconstitution.

In utilizing bone marrow for B cell reconstitution, effort will be made to evaluate or eliminate the presence of contaminating T cells.

Progress: New contract

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{2\ \&\ 3}{1\ \&\ 4}$)

Understanding the role of types and relative numbers of T and B cells in immune reactions is basic to manipulation of immune defense against cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunobiology Coordinating Committee
FY74 Funds: $17,000

CONTRACT RESEARCH SUMMARY

Title: Cell Synergy in Cell Bound Immune Reactions

Principal Investigator: Dr. Henric Blomgren
Name/Address Karolinska Institute
Performing S-104 01, Stockholm 60, Sweden
Organization:

Contract Number: N01-CB-33868
Starting Date: June 25, 1973 Expiration Date: March 1974
Goal: 1) To evaluate synergism between thymus cells and lymph node cells in graft versus host and tumor rejection reactions, and 2) to evaluate T cell tolerance after passage through allogeneic hosts.

Approach: Project 1 will evaluate the interrelations between thymocytes and lymph node cells in several situations as follows: a) synergistically in graft versus host responses, b) antagonistically in graft versus host responses, c) synergistic effects on the rejection of allogeneic tumors, and d) synergy in reactions between thymocytes and lymph node cells in the mixed lymphocyte reaction (MLR). In particular, the kinetics of regenerating thymus cells, their circulating characteristics, and their PHA responsiveness will be evaluated.

Project 2 will be a study of the development of immunologic tolerance in T cell populations passaged through allogeneic hosts. A second part of this project will be evaluation of the specific tolerance that can be seen in the donor bone marrow that is used to reconstitute lethally irradiated animals shortly after irradiation. In particular, the duration of tolerance will be evaluated and the question of whether bone marrow from such a chimera can be used to protect lethally irradiated mice syngeneic with the marrow will be examined.

Progress: New contract

Significance for Cancer Research (NCP Objective $\frac{3}{6}$ Approach $\frac{3 \& 4}{1 \& 4}$)
Understanding the role of types and relative numbers of T and B cells in immune reactions, including tolerance, is basic to manipulation of immune defense against cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $20,000

CONTRACT RESEARCH SUMMARY

Title: Effector Mechanisms in Tumor-Associated Immune Reactions

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institute
S-104 01, Stockholm 60, Sweden

Performing Organization:

Contract Number: N01-CB-33870
Starting Date: June 25, 1973 **Expiration Date:** March 1974
Goal: To study the mechanisms of B cell action in tumors in six different model systems: 1) the role of antibody effect in cytotoxicity, 2) Burkitt lymphoma and mouse lymphoma in nude mice, 3) human B cells in relation to Burkitt cell in culture, 4) human glioma patients' lymphocytes in relation to glioma cell lines, 5) Marek's disease, and 6) evaluation of human chronic lymphatic leukemia cells for capacity to act as cytotoxic cells.

Approach: 1) Evaluate humoral antibody effect on the cytotoxicity for tumor of several classes of lymphocytes in which the lymphocytes will be separated by methods worked out in conjunction with Dr. Wigzell. This work will be performed in a mouse system and due regard will be given for H-2 incompatibility of host and tumor. 2) Evaluate B cell capacity to reject tumors by utilizing nude (thymusless) mice. The tumors to be studied shall include human B cells to attack Burkitt lymphoma cells as a function of the lymphoma cells' coating of IgG. 4) Study lymphocytes derived from patients with glioma for the kinetics of the lymphocyte blastogenic response to glioma cell lines. 5) Evaluate tumor specific antigens, surface immunoglobulin, and vaccination phenomena in Marek's disease. 6) Study human chronic lymphatic leukemia cells (presumably B cells) for their capacity to act as killer cells against antibody coated target cells.

Progress: New Contract

Significance for Cancer Research (NCP Objective_3_Approach_4_)
6 1
6 4

Project Officer: Barbara Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $24,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Roles of Purified Cells and Antibodies in Cell-mediated Lysis

Principal Investigator: Dr. Hans Wigzell
Name/Address Karolinska Institute
S-104 01, Stockholm 60, Sweden

Performing
Organization:

Contract Number: N01-CB-33859
Starting Date: June 25, 1973 Expiration Date: March 1974

Goal: To evaluate several different proposed methodologies for the purpose of achieving high concentrations of T cells and B cells independently of each other.

Approach: The following methodologies will be thoroughly evaluated: 1) the use of antigen coated bead columns to cause adherence of B cells with the subsequent use of dextranase to dissolve the beads, 2) selective cytolysis of T cells with specific anti-T cell antibody, and 3) the evaluation of monolayers of appropriate target cells to achieve adherence of specifically sensitized lymphocytes.

For monitoring, at least three target systems will be used, chick red blood cells, tumor cells (human or mouse), and histoincompatible fibroblasts or tumor cells.

Progress: New contract

Significance for Cancer Research (NCP Objective 3/6 Approach 3 & 4/4)

The ability to work with purified populations of immunocompetent cells is basic to understanding and manipulating immune control of cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $19,000

CONTRACT RESEARCH SUMMARY

Title: Study of the Immune Responses of Mice and Rats to Tumor Associated Antigens

Principal Investigator: Dr. David Lavrin
Name/Address Litton Bionetics, Inc.
Performing 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: NIH-CB-23878
Starting Date: June 28, 1972 **Expiration Date:** April 27, 1974

Goal: Study of immune responses of mice and rats to tumor associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular and humoral immune responses.

Approach: The kinetics of the cellular and humoral immune responses to tumor associated antigens of the following tumors will be studied: tumors induced by polyoma virus, SV40, Gross and other murine leukemia viruses, murine sarcoma virus, and mammary tumor virus; mouse plasma cell tumors; methylcholanthrene-induced tumors. The relationship between the detected antigens and viral gene expression and expression of fetal antigens will be extensively studied.

The specificity of each of the detected antigens in both the humoral and cell-mediated immune studies are being intensively studied, by inhibition assays. The nature of the cells involved and the mechanism of cytotoxicity in the cell-mediated cytotoxicity assays are also being studied.

Progress: A major effort has been put into a continuing study of murine sarcoma virus (MSV)-induced cellular and humoral immunity in mice. Using transplantation and in vitro IAT, two main antigenicities have been demonstrated in SV40-induced tumors, a tumor associated surface antigen and a cross-reactive antigen. Kinetics of the SV40 TASA antibody response in mice have been determined using IAT and ^{51}Cr release. In vitro and in vivo studies of Freund leukemia antigens have been carried out. The effects of various immunosuppressive agents were also studied. The relative immunogenicity of fetal tissue and various DNA virus tumors to subsequent challenge with tumor cells was studied. Studies with mouse mammary tumors have been initiated. Other services provided have been maintenance of 4000-5000 mice and up to 150 rats and immunization of rabbits and mice, as desired by the Project Officer.

Significance for Cancer Research (NCP Objective 6 Approach 4)
Understanding of relationship of in vitro assays to host resistance to tumors is an essential element in properly using these assays to monitor response to immunotherapy.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology:Immunobiology **Site Visit Date:** Feb. 1974
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $160,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: In Vitro Tests for Tumor Specific Antigens, Antibodies and Immune Cells with Animal and Human Serum Cells

Principal Investigator: Dr. Edward Matthews
Name/Address Litton Bionetics, Inc.
Performing 7315 Wisconsin Avenue
Organization: Bethesda, Maryland 20014

Contract Number: N01-CB-02207
Starting Date: May 28, 1970 Expiration Date: April 27,1974

Goal: To prepare cultured melanoma cells for immunotherapy vaccine, to study development and function of cell mediated immune cytotoxicity in humans, and to assay viral infected human cell lines for leukemia associated antigens.

Approach: 1) Grow human melanoma cells free of PPL. Treat with neuraminidase and freeze under sterile conditions. Test for specificity and pyrogenicity. Provide for human immunotherapy. Also, adapt for culture explants of NCI melanoma patients. 2) Study direct cellular cytotoxicity of human lymphocytes. Evaluate lymphocyte dependent antibody with ^{51}Cr release assays. Immunize normal human peripheral blood lymphocytes in vitro. Study antitumor immunity in mice with progressively growing lethal tumors. 3) Murine leukemia viruses are used to infect human nonlymphoid cell lines. Cells are assessed for acute leukemia associated antigen. Cell membranes are solubilized and analyzed by gel electrophoresis.

Progress: Most of the melanoma cells requested by the Project Officers have been supplied in the form of RPMI-8072 vaccine. Seven rabbit anti-AKR leukemia antisera have been titered for cytotoxic lymphocyte dependent antibody and complement dependent antibody activity against AKR leukemia cells. MSV-M and MCA-induced tumors were established and tested for antibody in ^{51}Cr release assays. Studies of in vitro target cell damage by lymphocytes and humoral antibody were carried out. An extensive immunologic study was performed on purified plasma membrane from human tissue culture cells with antigens which cross react with HvALA.

Significance for Cancer Research (NCP Objective 6 Approach 1) Melanoma cell line material will be used for a clinical immunotherapy trial. In vitro immunization may boost an inadequate or blocked immune system. Leukemia associated antigen may be useful in finding etiologic agent for human leukemia.

Project Officer: Drs. William D. Terry, Dean L. Mann, John R. Wunderlich
Program: Immunobiology Site Visit Date: Feb. 1974
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $408,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunologic Studies of Murine RNA Oncogenic Viruses and Their Induced Tumors

Principal Investigator:	Dr. James McCoy
Name/Address	Litton Bionetics
Performing	Kensington, Md.
Organization:	

Contract Number: N01-CB-02320
Starting Date: June 29, 1972 Expiration Date: Dec. 28, 1974
Goal: Conduct immunologic studies of tumors induced by RNA oncogenic viruses and the immune responses they engender.

Approach: Newly induced neoplasms in inbred mice and hamsters are used following transformation _in vitro_ and _in vivo_ by MSV pseudotype viruses. Attempts are being made to evaluate the role played by new "cellular" antigens of the membrane or of viral antigens. Selection of "non-producer" neoplasms is being attempted. Comparison of the _in vivo_ immune responses to such antigens will be made with _in vitro_ colony inhibition, Cr^{51} release, proline release, microcytotoxicity assays.

The role of sensitized lymphoid cells and of antibody is being evaluated in the autochthonous host in the induction process of leukemia and of solid tumors using MSV and MSV(MLV).

Progress: Work during the previous contract year was conducted in close collaboration with the Project Officer in the study of tumor-associated transplantation antigens (TATA) of animal sarcoma virus-induced producer and non-producer tumor cells (releasing no infectious virus) and chemically-induced plasmacytomas of mice.

A variety of technical services were provided to the Project Officer in virological, immunological and biochemical studies of oncogenic viruses for providing membrane extracts of human tumors and animal tissues for skin sensitization studies. These studies are close to reaching a conclusion at the end of the present contract period and will then be terminated. Also, bulk produced SV40-induced mKSA cells and Visna virus-induced tumor cells were provided to the Project Officer for soluble antigen preparation.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Dr. Lloyd W. Law
Program: Immunology:Immunobiology Site Visit Date: Sept. 1973
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Revelance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $183,481

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation and Preservation of Plasma Cell Tumors in Mice

Principal Investigator: Dr. Kyle Sibinovic
Name/Address Litton Bionetics
Performing Bethesda, Maryland 20014
Organization:

Contract Number: N01-CB-92142
Starting Date: June 23, 1969 Expiration Date: Jan. 31,1975
Goal: Induction, transplantation and preservation of plasma cell tumors in mice.

Approach: Contractor preserve and transplants plasmacytomas in a liquid nitrogen bank and provides and ships these tumors on request to the NCI, and other investigators working with mouse plasmacytomas. In addition the contractor induces new plasmacytomas. Series of plasmacytomas producing antigen-binding myeloma proteins for the same hapten are being collected. In addition myeloma proteins from sources other than BALB/c are being collected. Contractor also assists project officer in the study of the mechanism of priming and tumor specific immunity to plasmacytomas. Contractor provides ascites containing myeloma proteins to NCI.

Progress: During the last contract period, Litton Bionetics, Inc. (LBI) maintained some 558 tumor lines including 154 new tumors which included the first tumors induced in the Bailey Recombinant Inbred mouse strains, CB20 congenic strains and those induced with MLV-A (Abelson virus) in BALB/c and Bailey Recombinant Inbred strains. A total of 161 lines were refrozen and evaluated to bring the number of ampules per line to the required level.

Significance for Cancer Research (NCP Objective 6 Approach 1)
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Project Officer: Michael Potter, M.D.
Program: Immunology:Immunobiology Site Visit Date:
Technical Review Group: Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $196,479

CONTRACT RESEARCH SUMMARY

Title: NCI Human Histocompatibility Typing Center

Principal Investigator: Name/Address Performing Organization:	Dr. R. J. Trapani Microbiological Associates, Inc. 4733 Bethesda Avenue Bethesda, Maryland 20014

Contract Number: NCI-CB-43915
Starting Date: June 16, 1969 Expiration Date: Nov. 1974
Goal: To assay solubilized HL-A alloantigens provided by the Project Officers and to study HL-A and other cell surface antigens on normal and neoplastic cells and their subcellular fractions.
Approach: The assay for solubilized HL-A alloantigen utilizes a cytotoxic inhibition assay. Target cells bearing the HL-A alloantigenic determinants to be analyzed are labeled with ^{51}Cr. The solubilized materials are incubated with the solubilized antigenic components and the inhibition of reaction of these antisera to the target cell demonstrates the presence of this antigenic material. Various serological tests, including cytotoxicity, C' fixation, and agglutination are used to assay HL-A and other antigens and human white blood cells and tissue culture cells both normal and malignant. Lymphocytes of patients with various malignant diseases are assayed to determine associations between these antigens and disease. Subcellular particles of human lymphoid cells are studied for presence of HL-A antigens.

Progress: The contractor has provided assays for the presence of HL-A alloantigenic materials provided by the Project Officer. In addition, heterologous antisera which precipitate these antigens have been raised by the contractor. The contractor has also begun looking at sera from patients with acute leukemia for the presence of antibody detecting an antigen associated with acute leukemia and/or antibody directed against HL-A alloantigens. Attempts are being made to differentiate and identify the development of antibody to acute leukemia associated antigens in patients with this disease. Several significant associations between HL-A antigens and disease categories had been found.

Significance for Cancer Research (NCP Objective 3 Approach 1,5)
Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor specific associated antigens.
Project Officer: Dean L. Mann, M.D., G. Nicholas Rogentine, M.D.
Program: Immunobiology Site Visit Date: Jul. 1973
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $196,000

CONTRACT RESEARCH SUMMARY

Title: Production of Human Lymphoid Cells in Culture

Principal Investigator: Dr. J. J. Oleson
Name/Address Pfizer, Inc.
Performing 199 Maywood Avenue
Organization: Maywood, New Jersey 07607

Contract Number: N01-CB-12078
Starting Date: January 1, 1971 Expiration Date: Dec. 1974
Goal: To grow and produce large quantities of human lymphoid cells in long term tissue culture and to grow the Raji cell line in human serum for immunization of acute leukemia patients.
Approach: Several human lymphoid tissue culture cell lines which bear different HL-A alloantigens are produced in large quantities and are isolated, concentrated, and shipped to the Project Officer for subsequent use in the isolation and characterization of the HL-A alloantigens. One of the cell lines, Raji, is being grown in quantities sufficient to initiate an immunization program in acute leukemia in humans. This cell line, unlike the other cell lines, is grown in normal human serum to eliminate the possibility of immunization with the fetal bovine serum foreign protein.

Progress: During the last contract period, 6999 grams of human lymphoid cells were supplied to the Project Officer. The production of Raji cells grown in human serum was increased significantly. Development of methods for membrane antigen extraction from lymphoid cells was begun.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Provides prototype technique for the isolation of tumor specific or tumor associated antigens. Raji cell line has been used for immunotherapy in acute leukemia.
Project Officer: Dean L. Mann, M.D., William D. Terry, M.D.
Program: Immunobiology Site Visit Date: June 1972
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: Waived per Dr. Berlin's memo of Feb. 15, 1973 to Dr. Terry
FY74 Funds $232,033

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune Related Cell Lines

Principal Investigator: Dr. Melvin Cohn
Name/Address The Salk Institute
Performing San Diego, California 92112
Organization:

Contract Number: N01-CB-23886
Starting Date: June 26, 1972 Expiration Date: Feb. 1975
Goal: To establish a library of lymphoma and myeloma tumor cell lines and make these available to investigators.

Approach: The contractor will catalog the available cell lines in his laboratory and characterize them as regards their lymphoma and myeloma characteristics. The results will be put into a catalog and either frozen cell lines or mice carrying the appropriate tumors will be made available to investigators on demand.

Progress: New items were developed to bring the catalog to the required 100 items, particularly immune related cells other than plasmacytomas. Over 180 catalogs were distributed and 159 shipments of cell lines and mice were sent out.

Significance for Cancer Research (NCP Objective 6 Approach 1) Experimental cancer immunology studies require cell lines and tumors of known characteristics. This contract provides a national resource which supports cancer research throughout the country.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Apr 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $83,153

CONTRACT RESEARCH SUMMARY

Title: Supramolecular Organization of Normal and Tumor Cell Surfaces and Their Relationship to Escape from Immune Surveillance and Growth Control

Principal Investigator: Garth L. Nicholson, Ph.D.
Name/Address The Salk Institute for Biological Studies
Performing P. O. Box 1809
Organization: San Diego, California 92112

Contract Number: N01-CB-33879
Starting Date: June 25, 1973 Expiration Date: Sept. 1974
Goal: To examine quantity and spatial organization of cell surface antigens and saccharides in normal and malignant cells.

Approach: Normal and transformed cells in tissue culture will be reacted with radioisotope labeled antibodies and lectins and then studied with electron microscopic and cell fractionation procedures.

Progress: New contract

Significance for Cancer Research (NCP Objective 3 Approach 5)
Understanding cell surface changes underlies many approaches to cancer prevention.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $111,420

CONTRACT RESEARCH SUMMARY

Title: The Use of Recombinant Inbred Strains of Mice for Analysis of Ir-1, Rgv-1 and Closely Linked Genes

Principal Investigator: Hugh O. McDevitt, M.D.
Name/Address Stanford University School of Medicine
Performing Stanford, California
Organization:

Contract Number: N01-CB-43941
Starting Date: March 1, 1974 **Expiration Date:** Feb. 1975
Goal: To develop and test mice bearing recombinant H-2 alleles in relation to analysis of the immune response.

Approach: The contractor will develop and maintain large breeding colonies of inbred mice for use in production and testing of antisera directed against a variety of known H-2 recombinants.

Progress: New contract

Significance for Cancer Research (NCP Objective 2 Approach 3)
Studies of genetically influenced immune responses are an important part of the baseline information flow of the Tumor Immunology Contract Program.

Project Officer: Barbara Sanford
Program: Immunobiology **Site Visit Date:** Feb 1974
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $122,000

CONTRACT RESEARCH SUMMARY

Title: Nature of Interactions Between Tumor Cells and Immunoglobulins

Principal Investigator: Dr. Isaac P. Witz
Name/Address
Tel Aviv University
Tel Aviv, Israel

Performing
Organization:

Contract Number: N01-CB-43868
Starting Date: August 15, 1973 Expiration Date: August 1974
Goal: This contractor will examine the role of natural and tumor specific immunoglobulin in coating of tumor cells in relation to antibody mediated and lymphocyte mediated cytotoxicity.

Approach: The contractor will (1) prepare purified Ig from acid eluates of coated tumor cells; (2) examine ascites, T lymphoma, mammary carcinoma, and carcinogen-induced sarcoma cells for complement-dependent cytotoxicity, lymphocyte-mediated cytotoxicity and antibody-mediated cytotoxicity, using uncoated tumor cells mixed with the acid eluates, in vivo coated tumor cells, and uncoated tumor cells as model systems; (3) utilize mice who have been immunized with noncross-reactive antigens and then challenged with ascites tumor cells and examine whether these cells show the capacity to bind the radiolabeled immunizing antigens and its effect on cytotoxicity assays; and (4) study the surface binding capacity of cultured tumor cells for various immunoglobulin fractions obtained from normal mice or from plasmacytoma bearing mice.

Progress: New contract

Significance for Cancer Research (NCP Objective 3 Approach 4)
The capacity of the immune system to recognize and eliminate transformed cells may depend on and be modified by immunoglobulins.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Control of Granulocyte and Macrophage Proliferation in Leukemia and Related Neoplasma

Principal Investigator: Donald Metcalf, M. D.
Name/Address The Walter and Eliza Hall Institute
of Medical Research
Performing Organization: Victoria 3050, Australia

Contract Number: N01-CB-33854
Starting Date: 20 June 1973 Expiration Date: 19 Oct. 1974
Goal: To examine the regulator substance known as colony stimulating factor (CSF) in relation to its formation, mechanism of action, and associations with cancer.

Approach: 1) Improve methods for producing the regulator substance Colony Stimulating Factor (CSF) and study its mode of action on target cells, both normal and neoplastic, 2) study the mechanisms by which antigens stimulate the formation of CSF, 3) evaluate the role of CSF in the regulation of granulopoiesis and monocyte formation in preleukemic and leukemic patients as well as normal humans and animals.

Progress: Newly approved contract.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Humoral factors, such as CSF, which regulate cells of the immune system are potentially powerful tools for immunotherapy.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Deferred
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $81,313

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator: Dr. G. J. V. Nossal
Name/Address
The Walter and Eliza Hall Institute
of Medical Research
Performing Organization: Victoria 3050, Australia

Contract Number: NO1-CB-23889
Starting Date: June 30, 1973 Expiration Date: Oct. 29, 1974

Goal: The work of this project is to analyze the relative roles of T and B cell mediated immune responses in the rejection of tumors in mice.

Approach: The approach will include an analysis of the mechanisms whereby tumor cells are killed and comparisons of various methods of inducing immune response against tumors. Plasma cell tumor systems will be utilized for at least some of the experiments, but fibrosarcomas, spontaneous reticulum cell sarcomas, parafin oil induced reticulum cell sarcomas, and other experimental tumors will also be studied. All will be examined from the point of view of establishing optimum methods for producing T cell immunity. A portion of this work will require biophysical separation methods that will be applied to permit separate study of T and B lymphocytes. Techniques presently in hand, as well as new procedures that are under investigation, will be applied to the separation of these cells and then the evaluation of the biologic function of the separated cells in tumor systems. The in vitro activation of T lymphocytes and an analysis of their killer activity will be undertaken. In addition, studies will be made of mechanisms for blockading killer cell activity. The final portion of the contract will deal with the mechanism of action of chemically defined adjuvants and their possible role in cancer immunology.

Progress: Two plasma cell tumors immunogenic in syngeneic mice have been selected from screening assays and established as cloned continuously cultured cells. After elimination of adherent cells, spleen cell suspensions have been found to be separable into B and T cell populations by electrophoresis. The tissue cultured plasma cell tumors have been found to be suitable targets for cell mediated immune assays in vitro and lymphocytes immunized in this way can inhibit tumor growth in vivo.

Significance for Cancer Research (NCI Objective 6 Approach 4)
Understanding and manipulation of T and B cell populations are crucial to immunotherapy of cancer.

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date: Waived 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $108,905

CONTRACT RESEARCH SUMMARY

Title: Multidisciplinary Cancer Research Center

Principal Investigator: Dr. Michael Feldman
Name/Address Weizmann Institute of Science
Rehovot, Israel

Performing
Organization:

Contract Number: N01-CB-23890
Starting Date: June 26, 1972 Expiration Date: May 25, 1974

Goal: To study the ability of various selected lymphoid cell populations to cause retardation of tumor growth in a carcinogen-induced tumor model; to adapt the bacteriophage plaque assay to detection of CEA and α-fetoprotein and to determine whether induction of a hyperthymic state in normal animals affects proliferation of transplanted tumors.

Approach: T and B cell populations will be sensitized against tumors and different T cell populations will be separately studied for their capacity to destroy tumor cells. Attempts will be made to remove potentially self-reactive lymphocytes before sensitization of these populations against syngeneic tumor cells. Various manipulations will be studied for effect on the growth of tumor cells in syngeneic systems. In the bacteriophage plaque assay, the contractor will purify CEA and α-fetoprotein antigens and explore various possibilities for attaching these to bacteriophage for the plaque assay. In thymus studies, a wide range of concentrations of syngeneic thymus cells or crude thymic humoral factors will be tested for ability to alter the proliferation of transplanted tumors.

Progress: Basic mechanisms of cell mediated tumor enhancement or rejection were investigated and a model for specific immunotherapy of metastatic tumor spread was developed. The isolation procedure for α-fetoprotein was scaled up so that larger amounts of protein could be obtained and affinity chromatography, utilizing an antibody-Sepharose column for isolation of protein, was employed. A synthetic approach was taken to preparation of CEA-modified bacteriophage. Experiments were carried out on in vitro cytotoxicity and in vivo tumor enhancement induced by mouse spleen cells autosensitized in vitro and studies on the mechanism of activation of lymphocytes by THF were undertaken.

Significance for Cancer Research (NCP Objective 5,6 Approach 4,1)
Specific cell populations will have to be appropriately manipulated to take maximum advantage of the potentialities of immunotherapy.

Project Officer: Drs. B. H. Sanford, T. Borsos, J. R. Wunderlich, H. J. Rapp
Program: Immunobiology Site Visit Date: Nov. 15-16, 1972
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $425,000

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. J. Claude Bennett
Name/Address University of Alabama in Birmingham
Performing Birmingham, Alabama 35294
Organization:

Contract Number: NO1-CB-43892
Starting Date: In negotiation Expiration Date:
Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: Using the TL antigens system of the mouse, the contractor will prepare and purify cell surface antigens and perform relevant biochemical studies. An attempt will be made to develop methodologies with wide applicability in studies of cell surface macromolecules.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $99,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Frank Lilly, Ph.D.
Name/Address Performing Organization: Albert Einstein College of Medicine
Bronx, New York 10461

Contract Number: N01-CB-43934
Starting Date: In negotiation Expiration Date:
Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity.

Approach: The contractor will study various strains of mice for responsiveness to H-2 D.2 antigen. Congenic strains will be used to identify H-2 linked and H-2 independent genes involved in the response.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Immune Responses in Relation to Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Eli E. Sercarz University of California Los Angeles, California 90024

Contract Number: NO1-CB-43972

Starting Date: In negotiation Expiration Date:

Goal: To identify single genes and/or polygenic systems involved in immune responses and to determine their relationship to tumor immunity.

Approach: The contractor will study the mechanisms by which the immune response to gallinaceous lysozymes is regulated at the genetic level in the mouse.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $80,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Development or Improvement of Techniques for In Vitro Sensitization of Human Lymphocytes

Principal Investigator: Name/Address Performing Organization: Paul Terasaki, Ph.D.
University of California
Los Angeles, California 90024

Contract Number: N01-CB-43884
Starting Date: In negotiation **Expiration Date:**

Goal: To develop improved methods for in vitro sensitization of human lymphocytes, directed at producing high yields of cytotoxic cells.

Approach: The contractor will evaluate the efficacy of various immunization methods, measuring release of ^{51}Cr from cultured human tumor cells. Variables will include target cell types, treatment of immunizing cells, types of responding cells, adjuvants, and culture conditions.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Donald A. Rowley, M.D.
Name/Address University of Chicago
Performing Chicago, Illinois 60637
Organization:

Contract Number: N01-CB-43998
Starting Date: In negotiation Expiration Date:

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will prepare anti-receptor antibody (ARA) and measure the effect of ARA on immune responses.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobioloby Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Jack H. Stimpfling
Columbus Hospital
Great Falls, Montana 59401

Contract Number: N01-CB-43935
Starting Date: In negotiation Expiration Date:

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will produce and distribute to other qualified investigators up to 100 mice per month of at least 5 different H-2 recombinant strains of mice.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $28,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Detection of Antigen - Binding Activity of Transplantable T-Cell Tumors

Principal Investigator:	Oliver A. Roholt, Ph.D.
Name/Address	Health Research Inc.
Performing	Roswell Park Memorial Institute
Organization:	Buffalo, New York 14203

Contract Number: N01-CB-43924
Starting Date: In negotiation Expiration Date:

Goal: To develop animal models of transplantable T-cell tumors possessing known specific antigen binding capacity.

Approach: The contractor will perform a pilot study, screening a minimum of 15 transplantable T-cell tumors for specific antigen-binding sites. The technique used for detection of antigen binding will be the paired iodination procedure.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $15,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: V.E.Olavi-Makela
Name/Address Helsinki University
Performing Helsinki, Finland
Organization:

Contract Number: N01-CB-43923
Starting Date: In negotiation Expiration Date:

Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study the fine specificity of various manifestations of acquired immunity and will also undertake studies on genetically controlled differences in fine specificity of anti-NP antibodies.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $17,500 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Production and Distribution of H-2 Recombinant or Mutant Congenic Strains of Mice

Principal Investigator: Marianna Cherry, Ph.D.
Name/Address Jackson Laboratory
Performing Bar Harbor, Maine 04609
Organization:

Contract Number: N01-CB-43988
Starting Date: In negotiation Expiration Date:

Goal: To facilitate the development of suitable production and distribution centers to provide H-2 recombinant or mutant congenic mice in adequate numbers for immunogenetic studies.

Approach: The contractor will make available for distribution mice of 8 H-2 recombinant congenic strains, 3 H-2 mutant congenic strains and 3 inbred partners of the congenic strains. Genetic testing will be carried out within lines and between appropriate lines to screen for recent mutations.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $28,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-Binding Receptors on T-Cells

Principal Investigator: Dr. Paul H. Maurer
Name/Address
Performing
Organization: Jefferson Medical College of Thomas Jefferson University
Philadelphia, Pennsylvania 19107

Contract Number: N01-CB-43999
Starting Date: In negotiation Expiration Date:
Goal: To investigate the properties of antigen-binding receptors on antigen-activated T lymphocytes.

Approach: The contractor will study binding of T cells and B cells with cross-reacting immunogens from immune responder mice, immunized non-responder mice and appropriate control non-immunized mice.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms for Cell Mediated Destruction of Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing Baltimore, Maryland 21218
Organization:

Contract Number: N01-CB-43995
Starting Date: In negotiation Expiration Date:
Goal: To study early cell surface changes associated with destruction of tumor target cells.

Approach: The contractor will evaluate the protein synthetic requirements for cytolysis, studying the effects of various inhibitors and the kinetics of the efflux of material of various molecular sizes from damaged target cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Dr. Michael Edidin
Name/Address Johns Hopkins University
Performing Baltimore, Maryland 21218
Organization:

Contract Number: N01-CB-43922
Starting Date: In negotiation **Expiration Date:**
Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will fuse mouse and human cells of normal and transformed lines and study antigen mobility.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $52,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: Dr. Saul Roseman
Name/Address Johns Hopkins University
Performing Baltimore, Maryland 21218
Organization:

Contract Number: N01-CB-43985
Starting Date: In negotiation Expiration Date:
Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate cell surface components involved in intercellular adhesion, prepare antibodies against these specific molecules and use such antibodies in studies of effects on the adhesive process *per se*, time of appearance of antigens during embryonic development and relationship with other cell surface components.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $63,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Human Tumor - Associated Antigens and Corresponding Antibodies

Principal Investigator: Dr. Eva Klein
Name/Address Karolinska Institutet
Performing Stockholm, Sweden
Organization:

Contract Number: N01-CB-43921
Starting Date: In negotiation **Expiration Date:**
Goal: To learn more about antigens and antibodies related to malignant transformation, for potential applicability to development of diagnostic tests and theraputic approaches for cancer.
Approach: The contractor will attempt to identify and characterize tumor associated antigens and antibodies in sarcoma, carcinoma and glioma patients. The immunoglobulin coating of tumor cells will also be studied.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $44,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Serologic and Immunogenetic Investigations of Tumor Associated and Normal Cell Surface Antigens

Principal Investigator: Ian McKenzie, M.D., Ph.D.
Name/Address Melbourne University
Performing Victoria, Australia
Organization:

Contract Number: N01-CB-43925
Starting Date: In negotiation Expiration Date:

Goal: To develop new test systems and serologic reagents for detection and characterization of cell surface antigens relevant to tumor immunobiology.

Approach: The contractor will study Ly-4.2 in normal and tumor tissues and will determine the correlation of Ly4.2 with other B cell markers. Studies of other Ly antigens will also be undertaken.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group. Tumor Immunology Coordinating Committee
FY74 Funds $39,550 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Improved Detection and Development of H-2 Recombinant Strains

Principal Investigator: Dr. Donald C. Schreffler
Name/Address University of Michigan Medical School
Performing Ann Arbor, Michigan
Organization:

Contract Number: N01-CB-43936
Starting Date: In negotiation **Expiration Date:**

Goal: To develop improved methods for studying mutation and recombination within the H-2 locus of the mouse, as a model for studying membrane changes in carcinogen or virus induced cancers.

Approach: The contractor will develop an extended series of H-2 recombinant or mutant congenic strains developed from a few key haplotypes. Screening will be done initially by serologic techniques, skin grafting and MLR, with such techniques compared to establish the most effective methods.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $74,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator: Dr. John W. Kreider
Name/Address Milton S. Hershey Medical Center
Performing Hershey, Pennsylvania
Organization:

Contract Number: N01-CB-43931
Starting Date: In negotiation Expiration Date:

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will quantitate and identify immunocytes in tumor mononuclear infiltrates, relating the ratio of lymphocytes of all types to tumor cells to *in situ* rates of death and proliferation. Immunocyte functional activity will be assessed *in vivo* and *in vitro*.

Progress: New contract.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group Committee on Cancer Immunobiology
Relevance Review Group Tumor Immunology Coordinating Committee
FY74 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Yves Borel
New England Medical Center
171 Harrison Avenue
Boston, Massachusetts 02111

Contract Number: N01-CB-43970
Starting Date: In negotiation **Expiration Date:**

Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the interaction of lymphoid cells, their membrane receptors, and their specific antigenic determinants during the induction and maintenance of immunologic tolerance.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Ross S. Basch, M.D.
Name/Address Performing Organization: New York University Medical Center
New York, New York

Contract Number: N01-CB-43927
Starting Date: In negotiation Expiration Date:

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will attempt to isolate, identify and determine the state of maturation of thymin-sensitive pre-T cells and to establish the mechanism through which thymin acts to convert these to cells resembling intra-thymic T cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: John H. Schwab, Ph.D.
Name/Address University of North Carolina
Performing Chapel Hill, North Carolina
Organization:

Contract Number: N01-CB-43997
Starting Date: In negotiation **Expiration Date:**
Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study the effects on the immune response of defined components of group A streptococcal cells and determine whether these can affect tumorigenesis or tumor growth.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Investigations of the Nature and Function of Immune-Related Cells in Tumor Masses

Principal Investigator: Stephen W. Russell, DVM, Ph.D.
Name/Address Scripps Clinic and Research Foundation
Performing La Jolla, California 92037
Organization:

Contract Number: N01-CB-44001
Starting Date: In negotiation Expiration Date:

Goal: To provide information about the numbers of immune-related cells in tumor masses, their identification, function in the tumor mass and factors influencing their movement.

Approach: The contractor will undertake precise identification and quantitation of immune-related cells in neoplasms. An attempt will be made to develop improved methods for use in identification and quantitation of immune related cells in neoplasms and improved methods of tumor disaggregation.

Progress: New contract.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Project Officer Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group Committee on Cancer Immunobiology
Relevance Review Group Tumor Immunology Coordinating Committee
FY74 Funds $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Donald F. H. Wallach, M.D.
Name/Address Tufts - New England Medical Center
Performing 171 Harrison Avenue
Organization: Boston, Massachusetts 02111

Contract Number: N01-CB-44000
Starting Date: In negotiation Expiration Date:
Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will undertake fractionation and characterization of plasma membranes of normal and neoplastic lymphocytes, using normal and SV40 transformed lymphoid cells as a model. Membrane dynamics will be studied and an attempt will be made to establish the dynamic topology of normal and SV-40 transformed lymphocytes.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $99,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Mechanisms of Lymphoid Cell Differentiation

Principal Investigator: Paavo Toivanen, M.D.
Name/Address Turku University Medical School
Performing Turku, Finland
Organization:

Contract Number: N01-CB-43971
Starting Date: In negotiation Expiration Date:

Goal: To increase understanding of lymphoid cell differentiation, which is fundamental in investigations of immunologic responses to tumors.

Approach: The contractor will carry out studies on origin and differentiation sites of pre-bursal stem cells in chickens and on mechanisms of T cell differentiation during early ontogeny in man and sheep.

Progress: New contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $40,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator: Dr. E.D.Kiehn
Name/Address University of Washington
Performing Seattle, Washington 98195
Organization:

Contract Number: N01-CB-43987
Starting Date: In negotiation Expiration Date:
Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell lines transformed by wild type and mutant polyoma viruses in comparison to their normal counterparts, investigating regulation of growth levels in vitro and control of cell surface morphology.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $23,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Studies of the Mechanisms by Which Tumors Avoid Destruction by the Immune System

Principal Investigator: Dr. Nechema Haran-Ghera
Name/Address Weizmann Institute
Performing Rehovot, Israel
Organization:

Contract Number: N01-CB-43930
Starting Date: In negotiation Expiration Date:
Goal: To determine which mechanisms are important in preventing an immune response from effectively destroying tumor cells.

Approach: The contractor will investigate the mechanism of escape from controlled proliferation to overt leukemia development in C57 BL/6 mice.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $90,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Name/Address Performing Organization: Dr. D.C. Edwards
Wellcome Research Laboratories
Beckenham, Kent, England

Contract Number: N01-CB-43929
Starting Date: In negotiation **Expiration Date:**
Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will study means for selectively stimulating T cell responses to well-defined antigens. An attempt will be made to distinguish adjuvants which act primarily on T cells and others which act on B cells and to devise methods for linking these adjuvant molecules to antigens and to antibodies.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $16,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Oliver Smithies
University of Wisconsin
Madison, Wisconsin 53706

Contract Number: N01-CB-43986
Starting Date: In negotiation Expiration Date:
Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will work on development of improved methods of determining primary amino acid sequences, suitable for use in sequencing normal and tumor cell surface antigens. β_2 microglobulin and other model proteins will be employed.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $60,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Selective Stimulation or Suppression of Humoral or Cellular Immunologic Responses

Principal Investigator: Dr. Richard K. Gershon
Name/Address Yale University School of Medicine
Performing New Haven, Connecticut
Organization:

Contract Number: N01-CB-43994
Starting Date: In negotiation Expiration Date:
Goal: To facilitate studies of the mechanisms involved in immunologic responses to tumor cells by separating components of the response.

Approach: The contractor will attempt to identify and characterize subpopulations of lymphocytes with the capacity to suppress responsiveness of other lymphoid cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobioloby Site Visit Date:
Technical Review Group: Committee on Cancer Immunobioloby
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $70,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Organization and Dynamics of Cell Surface Membrane Components Relevant to Tumor Immunology

Principal Investigator:	Dr. Richard A. Rifkind
Name/Address	College of Physician and Surgeons of Columbia
Performing	University
Organization:	New York, New York 10032

Contract Number: N01-CB-43880

Starting Date: In negotiation Expiration Date:

Goal: To increase understanding of the organization and dynamics of macromolecular components of normal and malignant cell surfaces.

Approach: The contractor will study cell surface properties associated with viral transformation of erythroid precusor cells and the effects of such alterations of surface properties on differentiation and proliferation.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $70,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal INvestigator: Harold F. Dvorak, M.D.
~~Name/~~Address Massachusetts General Hospital
Performing Boston, Massachusetts 20014
Organization:

Contract Number: NO1-CB-43894
Starting Date: In negotiation Expiration Date:
Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate, purify and chemically characterize cell coat material of normal and activated macrophages, ascites tumor cells and normal and transformed fibroblasts.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Committee on Cancer Immunobiology
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $45,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry of Normal and Tumor Cell Surface Antigens

Principal Investigator: **Name/Address** **Performing** **Organization:**	Sen-itoroh Hakomori, M.D., Ph.D. University of Washington, Dept. of Pathobiology Seattle, Washington

Contract Number: N01-CB-43920

Starting Date: In negotiation Expiration Date:

Goal: To isolate, purify and chemically characterize cell surface antigens.

Approach: The contractor will isolate and chemically characterize "galactoprotein a" "ceramide X" and sialylgalactosyl or sialylgalactosaminyl protein showing specific label for tumors and prepare, purify and characterize antibodies directed against such surface labelled components.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Project Officer: Barbara H. Sanford, Ph.D.

Program: Immunobiology Site Visit Date:

Technical Review Group: Committee on Cancer Immunobiology

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $58,000 (Estimate)

CONTRACT RESEARCH SUMMARY

Title: Development of Immunodiagnostic Tests for Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Henry Weimer University of California at Los Angeles Los Angeles, California 90024

Contract Number: NO1-CB-43860
Starting Date: September 1, 1973 Expiration Date: August 31, 1974

Goal: To identify new tumor associated antigens in carcinomas of the colon, stomach, lung, breast, prostate, and bladder which might be useful for immunodiagnosis.

Approach: Extract antigens from these carcinomas and from comparable normal tissues and from fetal tissues and prepare heterologous antisera against each of the antigens. Antigens shall be purified and antisera rendered specific for tumor associated antigens. Radioimmunoassays shall be developed for the detection of each antigen in serum or urine.

Progress: New contract; progress report has not yet been received.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New and more specific immunodiagnostic tests for the common types of human cancer are needed.

Project Officer: K. Robert McIntire, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $162,155

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator:	Dr. Leonard Chess
Name/Address	Children's Cancer Research Foundation
Performing	35 Binney Street
Organization:	Boston, Massachusetts 02115

Contract Number: N01-CB-43964
Starting Date: In negotiation Expiration Date:

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Robert K. Oldham, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvement of Assays for Cell Mediated Immunity: Methods for Isolation and Preservation of Lymphocytes for Reproducible Reactivity in Immunological Tests

Principal Investigator:	Dr. Roy Weiner
Name/Address	Children's Cancer Research Foundation
Performing	35 Binney Street
Organization:	Boston, Massachusetts 02115

Contract Number: N01-CB-43885
Starting Date: In negotiation Expiration Date:

Goal: To develop improved techniques for the preservation of the immunological reactivity of human peripheral blood lymphocytes.

Approach: Lymphocytes will be separated from the peripheral blood of normal individuals and of patients with carcinomas and will be preserved in the frozen state and then tested for retention of their biological properties, to include reproducible immunological reactivity.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Robert K. Oldham, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Purified Carcinoembryonic Antigen and Antisera

Principal Investigator: Dr. Charles W. Todd
Name/Address City of Hope National Medical Center
Duarte, California 91010

Performing
Organization:

Contract Number: NO1-CB-23877
Starting Date: June 1, 1972 Expiration Date: May 31, 1975

Goal: To provide purified carcinoembryonic antigen and antisera to carcinoembryonic antigen for use at the NCI Immunoglobulin Reference Center (Contract No. NO1-CB-33875) in studies involving the immunodiagnosis of cancer.

Approach: The contract will use presently available techniques to purify carcinoembryonic antigen. In addition, the contractor will investigate additional approaches for the preparation and isolation of these materials. Purified antigen will be used to immunize animals and produce antisera of appropriate specificity and strength for use in radioimmunoassay for diagnostic purposes. Purified CEA will be partially characterized from the chemical point of view, and where indicated, fragments will be prepared and their structure determined.

Progress: Contractor has supplied antigen and antisera to a number of investigators and has conducted several training sessions for investigators interested in learning the technique. Continued production of CEA and supply of materials for collaborative efforts with NCI have been carried out. Studies substantiate the apparent chemical homogeneity of the protein portion of molecules carrying CEA activity.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Knowledge of the structure of CEA might make possible improved assays for detection of this carcinofetal antigen.

Project Officer: William D. Terry, M.D.
Program: Immunodiagnosis Site Visit Date: Feb. 18, 19, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $210,282

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays for Circulating Tumor Associated Antigens: Clinical Usefulness of Carcinoembryonic Antigen (CEA) Assay as an Adjunct in the Diagnosis of Gastrointestinal Cancer

Principal Investigator: Dr. Theodore Hersh
Name/Address Emory University
Performing 1364 Clifton Road, N.E.
Organization: Atlanta, Georgia 30322

Contract Number: N01-CB-43887
Starting Date: In negotiation Expiration Date:

Goal: To determine whether the CEA assay is a useful adjunct in the diagnosis of gastrointestinal cancer.

Approach: Patients who have signs and symptoms suggestive of gastrointestinal cancer will have complete clinical and laboratory evaluation, and in addition CEA values will be determined. Correlation of elevated CEA levels with initial diagnosis of gastrointestinal cancer or development of cancer on subsequent followup will be evaluated.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Thomas A. Waldmann, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $47,965

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Assays of Circulating Tumor Associated Antigens: Clinical Usefulness of the Alphafetoprotein (AFP) Test and Human Chorionic Gonadotropin (HCG) in the Differential Diagnosis of Testicular Cancer

Principal Investigator:	Dr. Charles L. Vogel
Name/Address	Emory University
Performing	1364 Clifton Road, N.E.
Organization:	Atlanta, Georgia 30322

Contract Number: N01-CB-43888

Starting Date: In negotiation Expiration Date:

Goal: To determine the clinical usefulness of alpha fetoprotein test and test for human chorionic gonadotrophin in the differential diagnosis of testicular cancer.

Approach: Patients with signs or symptoms consistent with the diagnosis of intestinal cancer will be evaluated clinically and will also have AFP and HCG tests performed. Correlation of the test results and diagnosis of cancer will be examined.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Thomas A. Waldmann, M.D.

Program: Immunodiagnosis

Technical Review Group: Committee on Cancer Immunodiagnosis

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $17,758

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Name/Address	Dr. Ariel Hollinshead
Performing Organization:	George Washington University 2300 I Street, N.W. Washington, D. C. 20037

Contract Number: NIH-NCI-G-69-2176
Starting Date: June 27, 1969 Expiration Date: February 28, 1975

Goal: To determine whether skin testing for delayed hypersensitivity reactions can be used to measure cellular immunity to tumor-associated antigens. To use the skin test technique as an assay for separation and purification of tumor antigens.

Approach: Extraction and purification of skin reactive antigens from carcinoma of the breast, malignant melanoma, and acute leukemia.

Perform skin tests on patients at various stages of disease, to determine relationship of reactivity to clinical course.

Provide antigens for possible use in in vitro assays of cell-mediated immunity.

Progress: In both carcinoma of the breast and in malignant melanoma, two separate skin reactive antigens have been identified. One is tumor associated and it has only produced positive delayed hypersensitivity skin reactions in patients with the particular tumor type. The other antigens are tissue associated and have been found in normal tissues as well as in the tumors. These latter antigens have shown broader reactivity in patients. In acute lymphocytic leukemia, skin reactive antigens have been partially isolated and have been shown to be present in fetal thymus cells as well as in leukemic blast cells.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A fundamental problem in tumor immunology is the demonstration of tumor specific antigens in human tumors. This contract provides an opportunity to study delayed skin reactions to antigens.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology:Immunodiagnosis Site Visit Date: January 3, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $137,900

CONTRACT RESEARCH SUMMARY

Title: Development of Animal Models for Circulating Tumor Associated Antigens Potentially Useful in the Immunodiagnosis of Carcinoma

Principal Investigator: Dr. James W. Osborne
Name/Address University of Iowa
Performing Iowa City, Iowa 52242
Organization:

Contract Number: N01-CB-43891
Starting Date: In negotiation **Expiration Date:**

Goal: Perform studies of circulating tumor associated antigens in animal carcinomas that would serve as a model for common human cancers and that are potentially useful in developing immunodiagnostic tests for cancer.

Approach: Study antigens associated with radiation induced intestinal tumors in mice. Demonstrate and isolate circulating tumor associated antigens and design specific tests for quantitating the antigen. Study the relationship of antigen appearance to pre-malignant lesions and determine the levels of antigen in relation to tumor size.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Chou-Chik Ting, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $84,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvements of Assays for Cell Mediated Immunity: Development of New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. Christopher S. Henney
Name/Address Johns Hopkins University
Performing 725 N. Wolfe Street
Organization: Baltimore, Maryland 21205

Contract Number: N01-CB-43965
Starting Date: In negotiation Expiration Date:

Goal: Development of new in vitro techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: In syngeneic animal tumor model systems, the use of ^{86}Rb will be compared with the release of ^{51}Chromium for measuring cytotoxicity of tumor cells by immune lymphocytes. The use of gluteraldehyde-fixed tumor cells for lymphocyte stimulation will be examined. The conjugation of soluble tumor antigens to sensitive indicator cells for measurement of cell-mediated immunity to tumor antigens will be examined.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $90,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: **Experimental Systems for Carcinoembryonic Antigen**

Principal Investigator: Dr. David M. Goldenberg
Name/Address University of Kentucky
Lexington, Kentucky
Performing
Organization:

Contract Number: N01-CB-33869
Starting Date: June 30, 1973 Expiration Date: March 31, 1975

Goal: To produce carcinoembryonic antigen (CEA) which will then be provided to other investigators for analysis of its structure and to provide cell lines which produce CEA for use in studies of cellular hypersensitivity in patients.

Approach: Will develop two human colon tumor lines maintained in hamster cheek pouches for the purpose of providing carcinoembryonic antigens in quantity to other investigators for analysis of the structure of the material. Will provide cell lines derived from the tumors carried in hamster cheek pouches for the purpose of studying cellular hypersensitivity in patients with various carcinomas.

Progress: Carcinoembryonic antigen(s) has been isolated and partly characterized from human colonic carcinomas serially propagated in the cheek pouches and hind leg musculature of hamsters. These tumor systems are now producing virtually unlimited quantities of a relatively uniform preparation of these antigens, the major immunoreactive molecule being of 500,000 dalton size. Results indicate that although there is an apparent molecular size difference in the CEA extracted from our human colonic tumor xenografts and CEA from human metastatic colonic cancer, they nevertheless are immunologically identical. A number of cell cultures have been established from our cheek pouch human tumor xenografts, and have been found to contain a number of cell types, ranging from CEA-producing human colonic tumor cells to hamster-like "transformed" fibroblasts. These various cell types have been subcultured and cloned for cytogenetic analysis and CEA determination.

Significance for Cancer Research (NCI Objective 5 Approach 4)
Structural analysis of CEA from various sources will improve understanding of its relationship to tumor origin and size.

Project Officer: William D. Terry, M.D.
Program: Immunodiagnosis Site Visit Date: None
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $43,606

CONTRACT RESEARCH SUMMARY

Title: Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator: Akira Kobata, Ph.D.
Name/Address Kobe University
Performing Ikuta-ku
Organization: Kobe, Japan

Contract Number: N01-CB-43886
Starting Date: In negotiation Expiration Date:

Goal: To define the nature of the antigenic determinants of carcinoembryonic antigen and other cellular glycoproteins.

Approach: Develop enzymatic methods for obtaining oligosaccharides with serological activity. Purify and characterize endoglycosidases that will act on CEA, blood group glycoproteins or other macromolecules containing carbohydrate antigenic determinants.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $50,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Detection by Immunoflourexcent Techniques of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. Fiorenzo Paronetto
Name/Address Mt. Sinai School of Medicine
Performing 5th Avenue at 100th Street
Organization: New York, New York 10029
Contract Number: N01-CB-43953 Starting Date: In negotiation
Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcinoembryonic Antigen and Alphafetoprotein

Principal Investigator:	Dr. Elliot Alpert
Name/Address	Massachusetts General Hospital
Performing	Boston, Massachusetts 02114
Organization:	

Contract Number: N01-CB-43889
Starting Date: In negotiation Expiration Date:

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $55,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for Evaluation of Cancer Detection Tests

Principal Investigator:	Dr. Heinz Wahner
Name/Address Performing Organization:	Mayo Foundation 200 First Street, S.W. Rochester, Minnesota 55901

Contract Number: NIH-NCI-G-72-3879

Starting Date: June 15, 1972 Expiration Date: November 14, 1974

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the tumor immunology-immunodiagnosis program.

Progress: A bank of specimens from over 1,000 patients has been established. Coded specimens have been distributed to a large number of investigators, for rapid evaluation of potential immunodiagnostic assays. Plans are now being made for enlarging the bank to act as the central repository of immunodiagnostic related sera.

Significance for Cancer Research (NCP Objective 5 Approach 4)
This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology:Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $55,180

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine

Principal Investigator: Dr. Roy Woods
Name/Address Meloy Laboratories, Inc.
Performing Springfield, Virginia 22151
Organization:
Contract Number: NO1-CB-33875 (formerly PH43-NCI-G-66-3)
Starting Date: November 26, 1965 Expiration Date: January 10, 1975
Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Qualitative and quantitative immunochemical techniques are used for the diagnosis of multiple myeloma, Waldenström's macroglobulinemia, agammaglobulinemia, and other diseases with immunoglobulin disorders. In addition, these same techniques are used to evaluate carcinoembryonic antigen and α-fetoprotein in the serum of patients with a variety of malignancies. Studies are carried out to classify the abnormal proteins of the abnormality diseases and to study the α-fetoprotein and carcinoembryonic antigens in relation to diagnosis, course of disease, and response to therapy. Appropriate reagents for the immunoglobulin and carcino-fetal antigen detections are prepared and evaluated. In addition, the Immunoglobulin Reference Center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation to qualified laboratories and in cooperation with the World Health Organization prepares and distributes international immunoglobulin reference serums. The Immunoglobulin Reference Center will also serve as a distribution point for carcino-fetal standard preparations when they are available. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.

Progress: CEA assays have been used to follow patients with metastatic disease during drug therapy. Preliminary data indicate that CEA fluctuates with, but does not precede, other measures of tumor load in patients with advanced cancer. Alphafetoprotein and human chronic gonadotrophin levels are also being determined on serums from patients with cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: W. D. Terry, M.D., M. Blaese, M.D., T. A. Waldmann, M.D.
Program: Immunodiagnosis Site Visit Date: November 19, 1973
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $340,223

CONTRACT RESEARCH SUMMARY

Title: Procurement of Tumor Specimens and Serial Serum Specimens from Cancer Patients

Principal Investigator: Eugene B. Rosenberg, M.D.
Name/Address
Performing Organization: University of Miami
Coral Gables, Florida 33124

Contract Number: NO1-CB-43861
Starting Date: August 15, 1973 Expiration Date: October 31, 1974

Goal: Collection of serum specimens from patients with malignant diseases over a period of time to be used for the development and evaluation of potential immunodiagnostic tests for cancer.

Approach: Serum and plasma specimens will be obtained from patients with carcinomas of the colon, lung, breast, prostate, bladder, and other malignant diseases and from patients with benign diseases. A minimum of 25 patients per year of each category will be obtained. Specimens will be obtained before any therapy and at weekly intervals for one month and every two to three months thereafter.

Surgical specimens of the above tumors to the extent of a minimum of 25 tumors of each type per year will be obtained under sterile conditions and in conjunction with normal control tissues from the same organ sites. Serum, EDTA, plasma, and heparinized blood from some patients may also be required. In addition, a certain amount of dissection of tumor tissue and preparation of single cell suspensions, with appropriate packaging for shipping fresh and frozen specimens will be done.

Progress: A series of logistical problems for satisfactory collection of the tumor and serum specimens have been satisfactorily worked out and the collection of the specimens is proceeding well. The contractor has already been able to provide over 30 tumor specimens to other investigators.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Readily available collection of sera obtained from patients during the course of therapy for their cancers are necessary for prompt evaluation of promising new immunodiagnostic techniques.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $143,700

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: John S. Najarian, M.D.
Name/Address
Performing Organization: University of Minnesota
Minneapolis, Minnesota 55455

Contract Number: N01-CB-33915
Starting Date: June 30, 1973 Expiration Date: April 30, 1974

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: Collection of serum specimens and clinical information from these individuals are proceeding well. Annual progress report has not yet been submitted.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $72,236

CONTRACT RESEARCH SUMMARY

Title: Assays for Immune Depression in Cancer Patients

Principal Investigator: Edmond J. Yunis, M.D.
Name/Address
Performing Organization: University of Minnesota
Minneapolis, Minnesota 55455

Contract Number: N01-CB-43853
Starting Date: August 1, 1973 Expiration Date: July 31, 1974

Goal: To develop quantitative assays for the depression of various aspects of the immune response in human cancer patients and for following the clinical course of cancer patients.

Approach: Various parameters of the immune response will be evaluated in patients having carcinomas of the lung, breast, colon, prostate and bladder. These assays will be potentially applicable to large-scale use and will have been previously shown to be depressed in some cancer patients or to be depressed in animals with experimental tumors.

Progress: The project has been satisfactorily set up and the work is proceeding well. The annual progress report has not yet been submitted.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Assays for evaluation of the immune responsiveness of given individuals may prove to be highly important in understanding cancer patients' susceptibility to their tumors.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date: May 3, 1974
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $202,500

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Henry Altschuler, Ph.D.
Name/Address Philadelphia Geriatric Center
Performing 5301 Old York Road
Organization: Philadelphia, Pennsylvania 19141

Contract Number: NO1-CB-33914
Starting Date: June 25, 1973 Expiration Date: October 24, 1974

Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on an annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: Collection of serum specimens and clinical information from these individuals are proceeding well. Annual progress report has not yet been submitted.

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens of the Gastrointestinal Tract Other Than Carcioembryonic Antigen and Alphafetoprotein

Principal Investigator: Dr. Peter H. Schur
Name/Address Robert B. Brigham Hospital
Performing Boston, Massachusetts 02120
Organization:

Contract Number: N01-CB-43858
Starting Date: In negotiation Expiration Date:

Goal: To search for new tumor associated antigens of the gastrointestinal tract, which may be more useful for early diagnosis than CEA or AFP.

Approach: Identify by immunological methods antigens which may be useful in the detection of carcinoma of the gastrointestinal tract. Employ these methods in screening both biopsy specimens and cell cultures of gastrointestinal tract tumors for new antigens. Characterize new antigens and demonstrate usefulness for large scale testing of patient populations.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: R. Raymond Gantt, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $47,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen and Related Tumor-Specific Antigens in the Diagnosis of Cancer and as an Adjuvant in the Management of the Cancer Patient.

Principal Investigator: Dr. Tsann Ming Chu
Name/Address
Performing Organization:
Roswell Park Memorial Institute
666 Elm Street
Buffalo, New York 14203

Contract Number: N01-CB-33858
Starting Date: June 25, 1973
Expiration Date: Oct. 24, 1974

Goal: To evaluate the usefulness of carcinoembryonic antigen (CEA) assay in several varieties of human cancer. It will be evaluated for its capacity to 1) monitor recurrence of disease and 2) to measure the effectiveness of therapy.

Approach: This will be done by 1) determining a baseline value on all new patients who may fit into one of these categories, 2) evaluating CEA levels at 4 and 14 days after surgery (with some selected patients having more assays), 3) additional testing will be done at 30 and 60 days and every three months thereafter. For patients receiving radiation therapy, CEA testing will be done at two-week intervals during the therapy and at monthly intervals thereafter. Patients on chemotherapy will be tested at weekly intervals for ten weeks and then monthly.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)
CEA is one of the more promising assays for evaluating effects of therapy on certain cancers.

Project Officer: William D. Terry, M.D.
Program: Immunodiagnosis
Site Visit Date: To be done in FY 74
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: No Funds; Annual Rate: $56,250

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Jorgen E. Fogh, M.D. Sloan Kettering Institute for Cancer Research 145 Boston Post Road Rye, New York 10580

Contract Number: NO1-CB-43854
Starting Date: August 8, 1973 Expiration Date: August 7, 1974

Goal: To provide human tumor materials needed for the performance of assays for cell-mediated immunity.

Approach: Tumor specimens from patients having the more common tumors, such as carcinomas of the colon, stomach, lung, breast, prostate, and bladder, will be obtained from local sources and utilized for the development of techniques suitable for the isolation of viable tumor cells in good yield separated from contaminating normal cells. Tissue culture techniques which favor the maintenance of high viability of tumor cells will be developed. Cells maintained in such culture will be characterized by morphology and as many other techniques as possible to document their tumor origin. Techniques will be developed for the slow freezing and retrieval of viable cells suitable for growth in tissue culture or for immediate use in immunologic assays.

Progress: The initial major effort on this project was to compile a complete listing of available tissue culture cell lines of the various tumor types and to accumulate the known characteristics on these cells. This is now up to date and has been provided to a number of investigators within the tumor immunology program. Limited numbers of these cell lines are being made available for immunological investigations. Work on further characterization of the available tissue culture cell lines is proceeding well. Efforts are also being made to establish new cell lines and to prepare frozen aliquots of short term cultures from the various types of carcinomas.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Studies of cell-mediated immunity towards specific tumors as well as a general phenomenon in cancer patients is an urgently needed area of development in tumor immunology.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date: October 31, 1973
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $125,632

CONTRACT RESEARCH SUMMARY

Title: Detection by Immunoflourescent Techniques of Antibodies Against Autologous Human Tumor Cells

Principal Investigator: Dr. George J. Friou
Name/Address University of Southern California
Performing 2025 Zonal Avenue
Organization: Los Angeles, California 90033

Contract Number: N01-CB-43881
Starting Date: In negotiation Expiration Date:

Goal: To identify specific antibodies in the sera of patients with carcinoma of the lung, breast, and colon.

Approach: Sera from cancer patients and from controls will be tested against autologous tumor cells by immunofluorescence assays with viable and fixed tumor cells. Sera for testing will be obtained before and at appropriate intervals after therapy. Specificity of observed reactions will be determined.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $71,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Identification and Purification of Endoglycosidases Capable of Releasing Oligosaccharides from Glycoproteins

Principal Investigator: Dr. Om Bahl
Name/Address State University of New York
Performing Buffalo, New York 14214
Organization:

Contract Number: NO1-CB-43989
Starting Date: In negotiation **Expiration Date:**

Goal: To define the nature of the antigenic determinants of carcinoembryonic antigen and other cellular glycoproteins.

Approach: Develop enzymatic methods for obtaining oligosaccharides with serological activity. Purify and characterize endoglycosidases that will act on CEA, blood group glycoproteins or other macromolecules containing carbohydrate antigenic determinants.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: Barbara H. Sanford, Ph.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $74,100 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Improvements of Assays for Cell Mediated Immunity: Development of New In Vitro Techniques to Evaluate Cell Mediated Immunity to Intact Tumor Cells

Principal Investigator: Dr. John T. Harrington
Name/Address Performing Organization: University of Texas Medical School
San Antonio, Texas 78284

Contract Number: N01-CB-43893
Starting Date: In negotiation Expiration Date:

Goal: To develop new in vitro techniques or substantial modifications of existing techniques for the evaluation of cell-mediated immunity to whole tumor cells.

Approach: Develop Agarose droplet modification of migration inhibition assay for application to detection of immunity to human tumor antigens.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $70,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human Peripheral Blood Mononuclear Cells

Principal Investigator: Dr. Hans Wigzell
Name/Address University of Uppsala
Performing Uppsala, Sweden
Organization:

Contract Number: N01-CB-43883
Starting Date: In negotiation Expiration Date:

Goal: Development of procedures for more effective and reproducible fractionation of mononuclear cells from human peripheral blood.

Approach: Peripheral blood from normal individuals and from patients with carcinomas will be studied. The following cell types will be isolated in suspension with high yields, purity, and viability: T lymphocytes, B lymphocytes, and monocytes. The isolated cells will be characterized for viability, morphology, retention of membrane markers, and retention of immunological functions.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $25,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Search for New Human Tumor Associated Antigens in Carcinoma of the Lung

Principal Investigator: Dr. Robert W. Veltri
Name/Address West Virginia University
Performing Morgantown, West Virginia 26506
Organization:

Contract Number: N01-CB-43890
Starting Date: In negotiation Expiration Date:

Goal: Search for new tumor associated antigens in carcinoma of the lung, which may be more useful in immunodiagnosis than currently available markers.

Approach: Identify by immunological methods antigens which may be useful in detection of carcinoma of the lung. Employ these methods in screening both biopsy specimens and cell cultures of lung tumors, characterize detected antigens and determine distribution of any new antigen or related antibody in patients with cancer, benign disease, and in normal controls.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: K. Robert McIntire, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $100,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Development of Animal Models: Early Detection of Antibodies to Carcinomas in Experimental Animals as a Model for Human Studies

Principal Investigator: Dr. Lionel Manson
Name/Address The Wistar Institute
Performing 36th Street at Spruce
Organization: Philadelphia, Pennsylvania 19104

Contract Number: N01-CB-43882
Starting Date: In negotiation Expiration Date:

Goal: Development of experimental animal systems for the early detection of antibody response to tumor associated antigens, as model for problem of antibody detection in patients with carcinomas and other malignant diseases.

Approach: Studies will be performed with primary, autochthonous carcinomas or with carcinomas in early transplant, in inbred strains of mice. A sensitive antiglobulin assay will be used to determine antibody levels.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: R. Michael Blaese, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $76,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Biochemistry and Diagnostic Use of Human Tumor Antigens

Principal Investigator: Name/Address Performing Organization:	Dr. Vincent Marchesi Yale University New Haven, Connecticut 06510

Contract Number: N01-CB-43859
Starting Date: August 8, 1973 Expiration Date: November 7, 1974

Goal: To isolate and purify glycoprotein antigens from human colonic tumors.

Approach: The contract will use various extraction methods to isolate glycoproteins from human colonic tumors and prepare specific antisera to each of the isolated antigens. The specificity of these antigens will be compared with carcinoembryonic antigen prepared by usual procedures. Glycoproteins extracted from primary colon tumors, metastatic tumors, serum, and from embryonic tissues will be examined.

Progress: Contract is in first year and no progress report has been received.

Significance for Cancer Research (NCP Objective 5 Approach 4)
New methods for extraction of glycoprotein antigens from human colonic tumors may provide reagents with better specificity for immunodiagnosis than CEA.

Project Officer: J. Frederic Mushinski, M.D.
Program: Immunodiagnosis
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $108,000

CONTRACT RESEARCH SUMMARY

Title: Irradiate Experimental Animals

Principal Investigator: Dr. William Kiker
Name/Address Armed Forces Radiobiological
Performing Research Institute
Organization: Bethesda, Maryland 20014

Contract Number: NCI-GLC(70)-53
Starting Date: April 6, 1970 Expiration Date: 6/30/74

Goal: To lethally radiate rhesus monkeys in preparation for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction (GVHR), the major complication of this form of transplantation.

Approach: Rhesus monkeys receive X-radiation delivered by a Maxitron X-ray generator, in dosages requested by the Project Officer.

Progress: The contractor has satisfactorily radiated rhesus monkeys at our request.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Bone marrow transplantation may allow more intensive cancer chemotherapy than is presently available. Transplantation of allogeneic immune competent cells (bone marrow is one category) may be important in cancer immunotherapy.

Project Officer: G. Nicholas Rogentine, Jr., M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $2500 (est)

CONTRACT RESEARCH SUMMARY

Title: Subcellular Fractions in Immunotherapy - Augmentation of Tumor-Specific Immunity with Ribonucleic Acid (RNA).

Principal Investigator: Peter John Deckers, M.D.
Name/Address

Performing Organization:
Boston University Medical School
80 East Concord Street
Boston, Massachusetts 02118

Contract Number: N01-CB-43849
Starting Date: In negotiation Expiration Date:
Goal: Development of reliable in-vivo immunotherapy model with immune RNA in laboratory animals.

Approach: Analysis of the variables associated with optimal extraction and administration of RNA will be studied in small animal models.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,)3,4
Subcellular fractions such as immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: Estimated $65,000

CONTRACT RESEARCH SUMMARY

Title: Development of Immunolatex Particles for Detection of T and B Cells

Principal Investigator: William Dreyer
Name/Address Performing Organization: California Institute of Technology
Pasadena, California 91109

Contract Number: N01-CB-43875
Starting Date: 3/2/74 Expiration Date: 3/1/75
Goal: Development of a simple histochemical assay for B and T cells.

Approach: Utilizing antibodies specific for mouse B and T cells, the investigator will develop immunolatex reagents specifically for use in conjunction with standard Romanovsky stains. Once the model system has been developed in mice, a human antiserum will be utilized for the development of readily applicable simplified B and T cell assay.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4,6 Approach 1,2,4)
Widespread availability of simplified B and T cell assays would be extremely valuable for monitoring immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $95,730

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients Without Detectable Disease After Removal of Tumor-Containing Lymph Nodes

Principal Investigator: Donald L. Morton, M.D.
Name/Address University of California, Los Angeles
Performing Los Angeles, California 90024
Organization:

Contract Number: N01-CB-43852
Starting Date: August 8, 1973 Expiration Date: August 7, 1974
Goal: Conduct a suitably randomized study to evaluate promising preliminary evidence that BCG therapy is useful in melanoma.

Approach: Patients with a diagnosis of melanoma who have proven involvement of regional lymph nodes and whose disease has been totally removed as far as can be determined are appropriately randomized into treatment groups with due regard for sex, age, and disease classification. The three treatment arms are: 1) no further therapy, 2) BCG by tine injection, 3) BCG and melanoma cells intradermally. Clinical studies necessary to establish the diagnosis and document the patient's general health status, tumor status, toxicity of therapy, time to relapse, and survival time are being made. Some patients' immunologic status is being followed with a variety of in vitro assays.

Progress: As of the time of the site visit on March 8, 1974, 23 patients had been entered into the protocol. This is a patient accrual rate appropriate to the workscope of the contract.

Significance for Cancer Research (NCP Objective 6 Approach 4)
This is a direct extension of important preliminary observations regarding immunotherapy in human melanoma. This contract will permit a suitably controlled randomized clinical trial which should resolve a number of questions about this type of immunotherapy.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: March 8, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $199, 173

CONTRACT RESEARCH SUMMARY

Title: Hybrid Tumor Cells: An Immunotherapeutic Agent

Principal Investigator: Robertson Parkman, M.D.
Name/Address Childrens Hospital Medical Center
Performing Boston, Massachusetts 02115
Organization:

Contract Number: NO1-CB-43876
Starting Date: In negotiation Expiration Date:

Goal: Evaluate hybrid cells formed by fusion of tumors with non-tumor cells as immunotherapeutic agents.

Approach: Syngeneic animal systems will be used to evaluate the capacity of cells formed by fusion of tumor cells with fibroblasts to alter morbidity and mortality of tumor bearing animals. In-vitro comparison of immune response to hybrid tumor cells, irradiated tumor cells and live tumor cells in normal, tumor bearing, and "carrier" immunized animals will be evaluated.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2,6 Approach 1,4)
This project will explore a promising new direction in cancer therapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $30,000 estimated

CONTRACT RESEARCH SUMMARY

Title: Human Lung Cancer: Evaluation of BCG Therapy

Principal Investigator: Robert H. Waldman, M.D.
Name/Address University of Florida
Performing College of Medicine
Organization: Gainesville, Florida 32601

Contract Number: N01-CB-43857
Starting Date: 8/15/73 Expiration Date: 11/14/74
Goal: Evaluate immunotherapy in cancer patients with a poor prognosis but in otherwise good health.

Approach: Newly diagnosed and previously untreated patients with oat cell and squamous cell carcinomas of the lung will be randomized with due regard for age, sex, and known variables for survival in the specific tumor. BCG will be administered by a schedule to be arranged with the Project Officer. All clinical studies necessary to establish the diagnosis and the extent of the cancer, as well as periodic documentation of the patient's level of health, tumor status, reaction to therapy, deviations from protocol, time to relapse, and survival time will be made. Certain specified immunologic tests will also be conducted.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Because of the possibility that immunotherapy offers a distinct additive to other kinds of therapy, there is an urgent need to evaluate this therapy in patients who are not yet compromised by the extent of their disease.
Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: 5/18/73
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $110,500

CONTRACT RESEARCH SUMMARY

Title: Subcellular Fractions in Immunotherapy.

Principal Investigator: Yosef H. Pilch, M.D.
Name/Address

Harbor General Hospital
Performing 1000 West Carson Street
Organization: Torrance, California 90509

Contract Number: N01-CB-43950
Starting Date: In negotiation Expiration Date:
Goal: Evaluation of xenogenenic immune RNA and study of immune RNA in small animal models.

Approach: Two small models, including one spontaneously metastasizing tumor, will be studied for optimization of immune RNA as an immunotherapeutic maneuver.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,3) 4
Subcellular fractions such as immune RNA may be relatively nontoxic and possibly highly specific approaches to cancer immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: Estimated $80,000

CONTRACT RESEARCH SUMMARY

Title: Studies on Methanol Extractable Residue (MER) of BCG.

Principal Investigator: David W. Weiss

Name/Address Performing Organization:
Lautenberg Center for General & Tumor Immunology
Hebrew University - Hadassah Medical School
Jerusalem, Israel

Contract Number: N01-CB-02208

Starting Date: 6/29/73 **Expiration Date:** 6/28/74

Goal: Study the immunotherapeutic effects of MER and its mechanism of action.

Approach: Evaluation of MER as a therapeutic agent combined with irradiation and/or chemotherapy in the prevention of metastatic tumors in mice and guinea pig models, including optimization of dose and schedule of MER. These studies will also be applied to spontaneous mammary tumors and to both mice and guinea pigs subjected to chemical ocncogenic agents, early in the induction of tumors and after palpable tumors have arisen. The mode of action of MER will be assayed with a number of different in-vitro techniques for cell mediated and humoral immunity, some of which will utilize tumor cells as specific targets. In addition, the possiblity that MER, BCG organisms, certain other subcellular components of BCG and a large variety of taxonomically unrelated bacterial pathogens may share common antigens with a number of different types of neoplastic cells is being investigated.

Progress: Joint treatment with MER and either irradiation or chemotherapy has been found useful against transplanted, rapidly developing solid neoplasms. Administration of MER alone has been found to cause a significant lowering in the incidence of pulmonary metastases in a transplantable pulmonary carcinoma of C57Bl mice following extirpation of the initial implant. Earlier studies suggesting that limited amounts of MER pretreatment and limited antigenic stimulation favor the expression of cellular reactivity have been confirmed. This is in addition to evidence that the action of MER is on the immunological apparatus in an area not dependent on the participation of T cells. A number of in-vitro assays for quantitative assessment of mechanisms of immunologic resistance under the influence of MER treatment have been developed.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Development of preclinical models for immunotherapy and evaluation of mechanism of action in such models is the basis for rational approaches to human immunotherapy.

Project Officer: Dorothy Windhorst, M.D.

Program: Immunotherapy **Site Visit Date:**

Technical Review Group: Committee on Cancer Immunotherapy

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $77,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: A study of the immunological stimulant properties of Amphotericin B

Principal Investigator: J. Russell Little, M.D.
Name/Address Jewish Hospital of St. Louis
Performing 216 South King's Highway
Organization: St. Louis, Missouri 63110

Contract Number: N01-CB-43872
Starting Date: 3/1/1974 Expiration Date: 2/28/75

Goal: To evaluate immunologic responses in animal models receiving Amphotericin B (AmB)

Approach: B and T cell responses will be evaluated in animals receiving AmB following immunizations with soluble hapten protein conjugates. Other aspects of the humoral and cellular immune response will also be evaluated in relation to SRBC administration. The rejection time of histoincompatible skin grafts and GVH will also be evaluated in reation to AmB. Macrophage responses and AmB effects on the immunosuppressed animal will also be evaluated.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1,4) The possibility of using simple chemicals for the stimulation of the immune response must be thoroughly investigated.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Reference Review Group: Tumor Immunology Coordinating Committee
1974 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Multi-test Device with Standardized Antigens to Assay Delayed Hypersensitivity Via the Skin Test

Principal Investigator: Gary L. Hein
Name/Address Lincoln Laboratories, Inc.
Performing Decatur, Illinois
Organization:

Contract Number: N01-CB-43954
Starting Date: In negotiation Expiration Date:
Goal: To develop a multi-test device to be used in studying skin test responsiveness in patients receiving immunotherapy.

Approach: Standardized viral, bacterial, and fungal antigens will be developed in concentrated form which will permit their use on a multi-puncture skin test device. The skin testing device will be constructed so that multiple antigens may be applied simultaneously in a highly reproducible manner.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6, Approach 1)
Skin test reactivity is of basic importance in evaluating patients' immunologic competence.

Project Officer: John Ziegler, M.D. and Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Advisory Group
FY74 Funds: (est., $119,000)

CONTRACT RESEARCH SUMMARY

Title: Conduct Bone Marrow Transplantation Studies

Principal Investigator: Dr. Clement Darrow
Name/Address
Performing
Organization: Litton-Bionetics, Inc.
7315 Wisconsin Avenue
Bethesda, Maryland 20014

Contract Number: N01-CB-02103
Starting Date: March 9, 1970 Expiration Date: 6/8/74

Goal: To establish a preclinical model for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction(GVHR). A subsidiary goal is to study monkey transplantation antigens.

Approach: Rhesus monkeys are lethally radiated and then given histoincompatible bone marrow. Their subsequent course is followed clinically, hematologically, bacteriologcially, virologically, chemically, and immunologically. MOdification of GVHR is attempted with antilymphocyte serum (ALS) prepared by various methods and given to the recipient pre- and/or post-grafting or used for in vitro incubation of the marrow prior to infusion.

Progress: The transplantation regimen has been optimized and optimally controlled by establishment of a large series of x-ray, x-ray autograft, ALS x-ray autograft, and x-ray allograft controls. The maximal safe dose of x-ray has been defined and the effect of marrow dose has been defined in the autograft system. It has therefore been possible to demonstrate conclusively in the ALS autograft system that ALS which is free of hemopoietic stem cell toxicity may be prepared. A system for assay of hemopoietic stem cell toxicity of future lots of ALS has been developed. It has been shown that the early deaths associated with bone marrow grafts occur independently of dose over a 20-fold range of marrow doses up to maximum doses achievable without sacrifice of the donor and over the range of immunosuppressive non-gut-toxic x-ray doses. A major histocompatibility system has been defined in the rhesus and is designated RhL-A. We define at least 16 antigens of this system.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Bone marrow transplantation may allow more intensive cancer chemo- or radiotherapy than is presently possible. Transplantation of bone marrow may be important in cancer immunotherapy.

Project Officer: G. Nicholas Rogentine, Jr., M.D., R. Graw, M.D., J. Neefe, M.D.
Program: Immunotherapy Site Visit Date: 3/1/74
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $300,000 (est.)

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. James McCoy
Name/Address Litton Bionetics, Inc.
Performing 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: N01-CB-23227
Starting Date : March 1, 1972 Expiration Date: August 31, 1974

Goal: To study the immune responses of cancer patients to tumor associated antigens, and to correlate results of different assays with each other and with clinical course of disease.

Approach: A series of immunological tests have been set up to evaluate and monitor the general immune competence of patients and their response against tumor associated antigens. These assays include: lymphocyte stimulation by mitogens, recall antigens and tumor extracts; rosette assays for enumeration of T and B cells; leukocyte migration inhibition assays with recall antigens and tumor extracts; delayed hypersensitivity skin tests with tumor extracts; and cytotoxicity assays against tumor cells (microcytotoxicity assay and ^{125}IUDR cytotoxicity assay). Each of these assays will be performed serially on patients with various carcinomas, melanoma and lymphoma, and on benign disease controls and normal individuals.

Progress: During this past year a major emphasis has been placed on standardizing each of the assay procedures and in minimizing day-to-day test variability. This has been helped by successful cryopreservation of lymphocytes for use in the rosette assays, cytotoxicity assays, and lymphocyte stimulation assay. The assay for T cells by formation of E rosettes has shown decreased numbers of T cells in the blood of many cancer patients and this has returned rapidly to normal after complete tumor removal. In the leukocyte migration inhibition assay, extensive studies have been performed with KCl extracts of carcinoma of the breast and of malignant melanoma and have shown specific inhibition of migration in the majority of cancer patients tested. Cytotoxicity assays, both visual microcytotoxicity and radioisotopic release, have shown that many normal individuals have as much or even more reactivity than cancer patients. Only infrequently has tumor specificity been demonstrated in the cytotoxicity assays. In the lymphocyte stimulation assay, it has been shown that allogeneic KCl tumor extracts produced stimulation not only of cancer patients but also of normal individuals, presumably due to the presence of histocompatibility antigens. Membrane and KCl extracts have been prepared for use in delayed hypersensitivity skin tests. The results are being correlated.

Significance for Cancer Research (NCP Objective 5 Approach 4)
This contract will provide a comparison between several assays of cell mediated immunity in tumor patients and provide information on the usefulness of these assays in immunodiagnosis of cancer and in following the clinical course of patients.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunology:Immunotherapy Site Visit Date: July 11, 1973
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: DCBD Contract Program Review Committee
FY74 Funds: $460,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Role of Circulating Tumor Antigens.

Principal Investigator: Charles F. McKhann, M.D.
Name/Address
Performing
Organization: University of Minnesota
Minneapolis, Minnesota 55455
Contract Number: NO1-CB-43946
Starting Date: In negotiation **Expiration Date:**
Goal: To study the effects of circulating tumor antigens in relation to tumor size and response to immunotherapy.

Approach: Antigen and antibody complexes in the circulation of normal and tumor bearing mice will be evaluated in relation to tumor size and in-vitro measurements of immune responsiveness. Effects of irradiation in this system will also be studied. Tumor antigen, antibody to tumor antigen, and antigen-antibody complexes will be studied for stimulation and blocking of stimulation of lymphocyte dependent antibody and cellular cytotoxicity.

Progress: New Contract

1,5; 3,4
Significance for Cancer Research (NCP Objective 2,6 Approach ____)
Kinetics of circulating tumor antigens may have a profound effect on proper design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: Estimated $79,000

CONTRACT RESEARCH SUMMARY

Title: Tumor Specific Transplantation Antigens in Solid Tumors

Principal Investigator: Charles F. McKhann, M.D.
Name/Address
Dept. of Surgery
University of Minnesota
Performing Organization: Minneapolis, Minnesota 55455

Contract Number: N01-CB-92061
Starting Date: 6/14/73 **Expiration Date:** 6/13/74

Goal: Evaluate the role of immunity in the success or failure of tumors and determine how best to manipulate the immune response to the benefit of the host.

Approach: Evaluate the immune response of tumor-bearing animals by developing an immunologic profile to assay different components of the immune response under a variety of conditions of host-tumor interactions. In particular the role of circulating tumor antigens and their relationship to the size of the tumor will be evaluated.

Progress: This contract was transferred to this program in this fiscal year. The investigator reports having developed a new and highly sensitive methodology for detecting circulating tumor antigen which has permitted him to recognize direct effects of tumor size on the host responsiveness in vivo.

Significance for Cancer Research (NCP Objective 2, 6 Approach 1, 5, 3, 4.)
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy **Site Visit Date:**
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $140,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Role of Macrophages in Tumor Immunology

Principal Investigator: Jon R. Schmidtke Ph.D./Robert D. Nelson Ph.D.
Name/Address

Performing Organization: University of Minnesota
Minneapolis, Minnesota 55455

Contract Number: NO1-CB-43948

Starting Date: In negotiation Expiration Date:

Goal: Develop assays and models for evaluation of macrophages in immunotherapy.

Approach: Syngeneic tumors in mice will be utilized to study the role of macrophage function in both the afferent and efferent phases of the immune response. Studies will include evaluation of phagocytosis, catabolism of tumor antigens, the capacity of macrophages to transfer immunogenecity, tumoricidal capacity of macrophages and study of MIF effects. Human macrophages will be evaluated with a number of in-vitro assays.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Evaluation of macrophage activity is important to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.

Program: Immunotherapy Site Visit Date:

Technical Review Group: Committee on Cancer Immunotherapy

Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: Est. $65,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer

Principal Investigator: Richard L. Simmons, M.D.
Name/Address University of Minnesota
Performing Minneapolis, Minnesota 55455
Organization:

Contract Number: N01-CB-23885
Starting Date: June 30, 1972 **Expiration Date:** 1/31/75

Goal: The goal of this project is to test the usefulness of immunization with autochthonous, neuraminidase treated cells in the therapy of patients with widely disseminated human cancer not amenable to other techniques of treatment.

Approach: This will be done by (1) determining the immunologic competence of the patient, (2) determining the immunologic reactivity of the patient to his own and similar tumors, (3) instituting the trial of therapy with neuraminidase treated tumor cells derived from the patient, (4) redetermine the patient's immunologic competence at intervals during the course of treatment, (5) redetermine the patient's reactivity to his own tumor during the course of treatment (6)utilize the criteria of objective remission used by cooperative chemotherapy groups in following the tumors in these patients, and (7) therapy will be discontinued whenever lack of response is demonstrated. Patients will be limited to those with melanoma, fibrosarcoma, osteogenic sarcoma, lyposarcoma and neuroblastoma, who have been demonstrated to have residual tumor that is not amenable to further treatment with either chemotherapy or surgery. In addition, these patient will have been demonstrated to have good immunologic responses.

Progress:

Fifty-six patients were evaluated for inclusion in the study in the first 8 months. Nine died before therapy could be started. 48 patients have begun immunotherapy and 16 of these were still alive at the time of the report. Toxicity of BCG has been minimal and easily controlled. New protocols are now being developed for treatment of minimal residual disease.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Modified autochthonous tumor cells have been shown to be promising immunotherapeutic agents in animals. This contract attempts to apply the animal work to human cancer patients.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: 4/14/72
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73, $174,253 for 19 mos.)

CONTRACT RESEARCH SUMMARY

Title: Graft Versus Tumor: A Model for the Treatment of Carcinoma, Leukemia and Lymphoma

Principal Investigator: Mortimer M. Bortin, M.D.
Name/Address Mount Sinai Medical Center
Performing Milwaukee, Wisconsin 53233
Organization:

Contract Number: NO1-CB-33853
Starting Date: June 25, 1973 Expiration Date: August 24, 1974
Goal: To evaluate adoptive immunotherapy using histoincompatible immunocompetent cells in the treatment of spontaneous leukemia-lymphoma in AKR mice.

Approach: Using spontaneous tumors in AKR mice: 1) combinations of chemotherapy and chemoradiotherapy will be evaluated for treatment of leukemia and for inducing immunosuppression; 2) H-2 incompatible immunocompetent cells will be administered to the immunosuppressed animals and graft versus host reaction will be monitored; 3) chemotherapy and chemoradiotherapy plus antiserum against the H-2 incompatible cells plus immune H-2 compatible bone marrow will be administered for purposes of rescue from the graft versus host reaction.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)
Development of a usable animal model using donor immunocompetent cells with subsequent "rescue" from graft versus host disease could be a highly effective method of immunotherapy in humans.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73 - $70,200 for 14 months)

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: Dr. James F. Holland and Dr. J. George Bekesi
Name/Address Performing Organization:
Mt. Sinai School of Medicine
5th Avenue and 100th Street
New York, New York 10029

Contract Number: N01-CB-43879
Starting Date: In negotiation Expiration Date:
Goal: Evaluate Neuraminidase-treated allogeneic cells and MER in human myelocytic leukemia (AML).

Approach: Neuraminidase (VCN) treated allogeneic cells and MER will be evaluated in an appropriately designed, randomized study of AML patients after remission induction with cytosine arabinoside and Daunorubicin. Patients will be studied with both in-vivo and in-vitro immunologic tests.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1,4)
Suggestive early leads in immunotherapy of AML require that vigorous application of other possible modalities to this disease be undertaken.

Project Officer: Dorothy B. Windhorst, M.D.
Program: Immunotherapy Site Visit Date: 04/05/74
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $280,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Transfer Factor Therapy of Sarcoma

Principal Investigator: Albert S. LoBuglio
Name/Address Ohio State University
Performing Columbus Ohio 43212
Organization:

Contract Number: N01-CB-43878
Starting Date: April, 1974 Expiration Date:
Goal: Evaluate transfer factor as a therapeutic agent in human sarcomas.

Approach: Transfer factor will be derived by a chromatographic isolation technique from the leukocytes of relatives of tumor patients who have been shown to have tumor immunity with in vitro assays. Several different types of sarcomas will be studied in appropriately designed protocols.

Progress: New Contract

4 1, 2,3,4
Significance for Cancer Research (NCP Objective 2, Approach ___)
Transfer factor may be a relatively nontoxic and possibly highly specific approach to immunotherapy of cancer.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $133,323

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Development of Animal Models for Evaluation of Therapy with Neuraminidase Treated Tumor Cells

Principal Investigator: Name/Address Performing Organization:	G. Mark Kollmorgen, Ph.D. Oklahoma Medical Research Foundation 825 Northeast 13th Street Oklahoma City, Oklahoma 73104

Contract Number: N01-CB-43864
Starting Date: September 1, 1973 Expiration Date: October 31, 1974
Goal: To explore the parameters of therapy with neuraminidase treated autologous or isogeneic tumor cells.

Approach: Using known successful animal models, parameters of immunotherapy with tumor cells, such as cell number, route and schedule of administration, methods and duration of cell storage, and use of tissue culture cells, will be studied. In addition potential new model systems for evaluation of this form of immunotherapy will be explored. Such systems will be required to have high applicability to human cancer; that is, therapy will be evaluated in the presence of established tumors and the tumors will be of relatively recent origin.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)
Animal models for immunotherapy will not only contribute to the understanding of immunotherapy but also to the problems of diagnosis of the quantity of malignant disease and the evaluation of normal body mechanisms of defense against malignant disease.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: May 21, 1973
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: $128,350

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of Macrophages in the Immune Response

Principal Investigator: James L. Krahenbuhl, Ph.D.
Name/Address Performing Organization: Palo Alto Medical Research Foundation
860 Bryant Street
Palo Alto California 94301

Contract Number: N01-CB-43873
Starting Date: May 1, 1974 Expiration Date: 4/30/75
Goal: To study the role of macrophages in the immune response to tumors, particularly in relation to immunotherapy, both in animals and in man.

Approach: Guinea pig and mouse peritoneal macrophages and human peripheral blood monocytes and monocyte derived macrophages will be studies for their capacity to adversely affect a number of syngeneic and allogeneic tumor target cells. Kinetics of the response of activated macrophages will be examined and the macrophage activation related to acute and chronic stages of infection will be evaluated. Cell mediated immune responses of animals and patients to Toxoplasma antigens, streptokinase-streptodornase, and PPD will be evaluated by skin tests and by in vitro techniques.

Progress: New contract.

Significance for Cancer Research (NCP Objective 2,4,6 Approach 1,2,4)
Understanding mechanisms of macrophage activity has high relevance to the timing of immunotherapeutic manipulations.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committe on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $115, 412

CONTRACT RESEARCH SUMMARY

Title: Cancer Immunotherapy: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Gerald L. Bartlett, M.D., Ph.D.
Name/Address M. S. Hershey Medical Center
The Pennsylvania State University
Performing Organization: Hershey, Pennsylvania 17033

Contract Number: NO1-CB-33891
Starting Date: June 30, 1973 Expiration Date: October 31, 1974
Goal: To develop animal models for the assessment of the usefulness and parameters of administration of various types of immune stimulants as therapy for cancer.

Approach: Animal models with a direct applicability to human cancer will be studied for the effectiveness of various immunostimulants on their cancers. The immunotherapy will be given after tumor load has been reduced by surgery, radiation, or chemotherapy, and the tumor load will be quantifiable. Experimental variables will include strain of organism, dose, schedule, and route of administration.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1 &4)
Evaluation of parameters of promising forms of immunotherapy in animals is necessary for optimum utilization in humans.

Project Officer: William D. Terry, M.D./Berton Zbar, M.D.
Program: Immunotherapy Site Visit Date: May 30, 1973
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73 - $195,943 for 16 months)

CONTRACT RESEARCH SUMMARY

Title: Hapten Treatment of Cancer

Principal Investigator: Kazuhiko Arai, M.D.
Name/Address University of Pennsylvania
Philadelphia, Pennsylvania 19146

Performing
Organization:

Contract Number: NO1-CB-43967
Starting Date: In negotiation Expiration Date:
Goal: Evaluate L-phenylalanine plus antiserum in a syngeneic rat system.

Approach: Protocols utilizing rats which have previously indicated that intratumoral injection of L-phenylalanine mustard plus I.V. injection of antiserum against tumor-modified with L-phenylalanine mustard has a therapeutic effect will be used to study syngeneic rat tumors in vivo.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2, 6 Approach 1,4)

This work will evaluate a promising animal model in a system more applicable to human tumors

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $25,000 (Estimated)

CONTRACT RESEARCH SUMMARY

Title: Neuraminidase Effectiveness in Tumor Immunotherapy and Mechanism of Its Effect

Principal Investigator: Dr. Gabriel J. Gasic
Name/Address University of Pennsylvania
Performing Philadelphia, Pennsylvania 19104
Organization:

Contract Number: N01-CB-33860
Starting Date: June 25, 1973 Expiration Date: 8/24/74
Goal: To evaluate neuraminidase treated tumor cells in immunotherapy by establishing several animal tumor models and then examining the variables of dose, route of injection, etc., in these models. To examine the capacity of neuraminidase treatment in therapy of postsurgical metastatic cancer.

Approach: 1) Test the effectiveness of neuraminidase-treated tumor cells as immunotherapy in two spontaneous and three methylcholanthrene induced mouse tumors. 2) Use of model systems in which the neuraminidase treatment is effective, the following parameters will be evaluated: dose of treated tumor cells, numbers of successive subcutaneous injections, route of treatment, and time of administration after tumor grafting. 3) Evaluation of the effectiveness of neuraminidase-treated tumor cells in immunotherapy of metastatic tumor using metastatic tumor cells. 4) Examination of the effectiveness of neuraminidase modified cells in relation to the natural immunogenicity of certain target tumors.

Progress: New contract

Significance for Cancer Research (NCP Objective 6 Approach 1)
Manipulation of autologous tumor with neuraminidase is one of the more promising areas of tumor immunotherapy. Results of this work will have direct relevance to man.
Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73, $60,376 for 14 mos)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Accessible Neoplasms: An Analysis of Immune Status In Vivo

Principal Investigator: Dr. Edmund Klein
Name/Address Research Foundation of the State of New York
Performing Albany, New York (Roswell Park, Buffalo, N.Y.)
Organization:

Contract Number: N01-CP-33373 Previous Contract No. E-71-2137
Starting Date: 3/20/74 Expiration Date: 3/19/75

Goal: Exploratory studies in the utilization of topical immunotherapy and corrolation with in vivo measurement of immune status.

Approach: Patients with various neoplasms originating or metastasizing to the skin will be entered into specific clinical protocols for application of topical immunotherapeutic agents. Some patients will receive BCG by scarification. Multiple antigens will be used for skin testing in all patients and the skin reactivity will be correlated with clinical status and administration of immunotherapy.

Progress: This contract has recently been transferred and a new direction is being undertaken.

Significance for Cancer Research (NCP Objective 2,5,6 Approach 1,3,4)

Project Officer (s): Charles Boone, M.D. and Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
[illegible] Tumor Immunology Coordinating Committee
FY74 Funds: $73,496 (plus $9,000 from DCCP)

CONTRACT RESEARCH SUMMARY

Title: Macrophage Activation in Tumor Immunity and Immunotherapy of Rat Mammary Tumor

Principal Investigator: Dr. W. H. Churchill
Name/Address Performing Organization: Robert B. Brigham Hospital
Boston, Massachusetts

Contract Number: N01-CB-33896
Starting Date: 6/30/73 Expiration Date: 10/31/74
Goal: To study the mechanism of tumor immunogenicity in order to design a more effective and rational program of tumor immunotherapy in man.

Approach: The contractor will evaluate the ability of macrophages to kill tumor cells after in-vitro activation by lymphocyte mediators, and determine whether macrophages activated by adjuvants in-vivo show differences in tumor cell killing activity in-vitro. In addition, he will extend these studies to human monocytes.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 6 Approach 1,4)

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73 - $160,865 for 16 months)

CONTRACT RESEARCH SUMMARY

Title: Subcellular Fractions and Immunotherapy: Effect of Transfer Factor on Virus-Induced Tumors in Marmoset Monkeys.

Principal Investigator: Peter Baram, Ph.D.
Name/Address

Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
Chicago, Illinois 60612

Contract Number: N01-CB-43951
Starting Date: In negotiation Expiration Date:
Goal: Utilize a subhuman primate system to evaluate a promising mode of immunotherapy.

Approach: Two different virus-induced tumors of marmoset monkeys will be utilized to evaluate therapeutic effect of transfer factor, its specificity, and the optimum dose and schedule of administration. Transfer factor will be compared with immune RNA in some of these studies.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 2,4 Approach 1,2,)3, 4
Subcellular fractions such as transfer factor and immune RNA may be relatively nontoxic and possibly highly specific approaches to immunotherapy of cancer. Lack of suitable models in smaller animal forms requires that primate systems be studied.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date: April 29, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: Estimated $190,000

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of Circulating Tumor Antigens in Immunotherapy

Principal Investigator: Joseph D. Feldman, M.D.
Name/Address Scripps Clinic and Research Foundation
Performing 476 Prospect Street
Organization: La Jolla, California 92037

Contract Number: N01-CB-43874
Starting Date: 3/1/74 Expiration Date: 2/28/75
Goal: Evaluate the effects that circulating tumor antigens may have on immunotherapeutic maneuvers.

Approach: Several different antigens associated with a viral tumor system will be isolated. The metabolism of these antigens and the circulation of hosts with regressing and progressing tumors will be measured. The immune response to each category of antigen will be analyzed and the antigen will be utilized to evaluate their capacity to accentuate destruction of the tumor.

Progress: New Contract

Significance for Cancer Research (NCP Objective 2,6 Approach 1,5,)3,4,
Knowledge of how circulating antigens may influence immunotherapy is important for the design of courses of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $114,698

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Human Cancer

Principal Investigator: Jordan U. Gutterman, M.D.
Name/Address University of Texas
Performing M. D. Anderson Hospital
Organization: Houston, Texas 77025

Contract Number: NO1-CB-33888
Starting Date: June 25, 1973 Expiration Date: October 24, 1974
Goal: Evaluate BCG as a form of immunotherapy in several types of local and disseminated human cancer.

Approach: Patients with wide-spread involvement of a given cancer in relatively good condition but with a uniformly poor prognosis will be randomized with due regard for age, sex, general condition, and other variables determined by the particular tumor. Statistical considerations to insure evaluable results will enter into the randomization and the determination of patient numbers to be entered into protocols.

Progress: At the time of the site visit, approximately 200 patients had been entered into some 9 different protocols (6 different tumors) under this contract. In addition, 103 patients were being followed who had been entered into protocols prior to initiation of the contract.

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)
Because of the wide-spread casual application of immunotherapy to human patients, it is important that well-designed prospective ethical clinical protocols be established as soon as possible.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: March 26, 1974
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee

FY74 Funds: 0 (FY73, $463,000 for 16 months)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigator: Dr. Karl Erik Hellstrom
Name/Address University of Washington
Performing Seattle, Washington 98195
Organization:

Contract Number: N01-CB-23887
Starting Date: June 30, 1972 Expiration Date: Sept. 30, 1974

Goal: The goal of this project is to evaluate the use of "deblocking" serum as well as immunization with autochthonous tumor cells as possible therapeutic approaches in the treatment of malignant melanoma. BCG may also be used.

Approach: Preliminary studies will be carried out to assure that neither of the immunotherapuetic maneuvers appears to have toxic affects on recipients. After this has been established, a double-blind immunotherapy trail will be established wherein patients will be assessed to determine whether or not their serum contains blocking antibodies in the colony inhibition assay. Patients with blocking antibodies will be randomized into a test group which will receive deblocking plasma and a control group which will receive normal plasma. Patients whose serum does not contain blocking factor will be randomized into a treatment group that will receive a course of immunotherapy involving active immunization with intradermal inoculations of tumor cells mixed with living BCG, and a control group which will receive no additional therapy. A variety of laboratory investigations will be carried out on these patients.

Progress: The plasma of approximately 50% of normal Negroblood donors has been found to contain "deblocking" activity in cultures with melanoma patients tumor, serum, and lymphocytes. This provides a large enough source of plasma to treat a study group of melanoma patients. A phase I study has been completed and the approach is ready for randomized trial. BCG may be added to the therapeutic regimen. Neuroblastoma patients will not be studied because of insufficient numbers.

Significance for Cancer Research (NCP Objective 6 Approach 4)
This contract will provide for indepth laboratory analyses of patients' immune response during immunotherapy and coordination of these responses with clinical course.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: 3/26/73
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: 0 (FY73, $191,828 for 15 mos.)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Mechanism of Action of Immunopotentiators

Principal Investigator: Alan M. Kaplan
Name/Address Performing Organization: Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Contract Number: N01-CB-43877
Starting Date: April, 1974 Expiration Date: March, 1975
Goal: Evaluate the immunologic effects of immunopotentiators in relation to their immunotherapeutic capacity.

Approach: The immunologic action of selected immunopotentiators will be evalutated in model systems for antibody production and cellular immunity. The changes in these responses towards tumors from tumor bearing mice will be measured and compared with the model system. In addition, the effectiveness of the immunopotentiators in the treatment of tumors after using surgery or chemotherapy to remove the bulk of the tumor will be evalutated.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4,6 Approach 1,3,4, and 6.
Understanding the mechanism by which immunopotentiators act is necessary for optimum manipulation of immunotherapy.

Project Officer: Dorothy Windhorst, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $89, 800

CONTRACT RESEARCH SUMMARY

Title: Development of Animal Model for Evaluation of Therapy with Neuraminidase Treated Tumor Cells

Principal Investigator: Name/Address Performing Organization:	Dr. R. K. Gershon Yale University- New Haven, Conn. 06510

Contract Number: N01-CB-43865
Starting Date: 8/8/73 Expiration Date: 9/7/74

Goal: A systematic evaluation of various animal tumors for use in testing of therapy with neuraminidase treated tumor cells

Approach: Tumor models closely resembling human tumors will be established by various well known means. Neuraminidase and other glycosidases will be tested to determine if they have any effect on the capacity of tumor vaccines to effect tumor regression. Dosage and route of administration of such vaccines will be maximized. Nonviable tumor cells and tumor cell fraction vaccines will be evaluated. Combination therapy with vaccine and other modalities will also be pursued.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)
Preclinical models of immunotherapy are highly important for optimizing human protocols.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Coordinating Committee
FY74 Funds: $113,712 (15 mos.)

ANNUAL REPORT

THE BREAST CANCER PROGRAM COORDINATING BRANCH

AND THE BREAST CANCER TASK FORCE PROGRAM

July 1, 1973 - June 30, 1974

INTRODUCTION AND SUMMARY

The Breast Cancer Task Force has continued to implement and extend the program first outlined three years ago in its "Detailed Research Logic" plans. These plans indicated a large number of tasks and research problems in the detection, treatment and prevention of breast cancer, and "baseline" studies in clinical fields, in epidemiology and in experimental biology. Responsibility for developing program in these areas was divided among four new technical review groups: the Breast Cancer Diagnosis Committee, the Breast Cancer Epidemiology Committee, the Breast Cancer Experimental Biology Committee, and the Breast Cancer Treatment Committee. Staff backing was provided by the Breast Cancer Program Coordinating Branch, and appropriate liaison and joint activities were set up with groups in other divisions of the National Cancer Institute.

Last year it was reported that the Breast Cancer Task Force Program had grown from 21 contract projects costing approximately $2.0 million at the end of June 1972, to 63 projects estimated at $5.7 million at the end of June 1973. This increase was the culmination of almost two years' effort to seek out new resources, through competetive procedures based on advertised requests for proposals. By the end of June 1974 the Program is expected to include some 70-75 projects totalling $7.1 million. Thus, the current year has been one of continuing but slower growth, reflecting (1) the opening of fewer new areas than in the previous year, (2) the phase-out of a few completed projects and (3) the continuation of most of the projects that were initiated during the past few years.

THE BREAST CANCER DIAGNOSIS PROGRAM

The Breast Cancer Diagnostic Committee has exerted its efforts this year to expanding its program in three research areas: detection, immunology and diagnosis-prognosis. In addition it has initiated a project to clarify the association of fibrocystic disease with breast cancer and is planning a study to develop diagnostic criteria for a standardized histopathologic classification of preneoplastic lesions.

In the detection area three contractors are engaged in the development and refinement of physical instrumentation for detection. One is building an ultrasonic scanner which will soon be available for clinical testing, another is perfecting electronic mammography techniques and a third investigator is designing an experimental multi-element infrared scanning system to be clinically tested for use in a mass screening program for breast cancer.

An inter-institutional study comparing the [illegible] of xeromammography versus film mammography will soon be initiated. [illegible] will assume vital importance in light of the Breast Cancer Demonstration Centers now becoming established throughout the country. In further effort to define early diagnostic criteria utilizing human material, the following investigators are actively involved in securing samples of breast secretions for biochemical, virological and cytological evaluation.

In the immunology area five contracts are working with three broad objectives. The first is to assure a supply of human breast cancer tissue for immunodiagnostic studies. For this purpose, one contract has been extended for another year. Secondly, long term human breast cancer cell lines are to be cultured and maintained. Two investigators involved in this effort have been successful in the growth and isolation of normal epithelial cells and tumor [illegible] work will proceed toward the precise characterization of these cell lines. Finally, two contractors are engaged in the development of immunological assays for detection of human breast cancer-associated antigens. One objective is to isolate specific breast tumor antigen and by immunochemical studies to define its immunochemical properties, its distribution and its potential use for serological diagnosis of populations at high risk. Another objective is to determine whether an immune response exists in breast cancer patients to tumor antigens associated with their own as well as allogeneic breast tumors.

In the area of diagnosis-prognosis, studies are being brought to completion which were aimed at the ascertainment of discriminants which would serve as prognostic and therapeutic indices. These involved the assessment of several clinical data, nodal involvement and the hormonal status of the host. The accumulated data are now undergoing statistical evaluation. A new study is just getting under way which will investigate steroid sulfate blood levels as possible diagnostic and/or prognostic parameters in breast cancer, by radioimmunoassay techniques in case-controlled populations. This would serve as another step in the continued search for biochemical markers useful for identification of population at high risk.

As seen at present, the Breast Cancer Diagnosis program will involve [illegible] of the approximately $7.0 million FY 1976 budget of the Task Force, as compared with 23.4% of the $5.2 million expended in FY [illegible].

BREAST CANCER EPIDEMIOLOGY PROGRAM

Since its inception, the Breast Cancer Epidemiology Committee has utilized and in some cases supported programs in viral etiology and hormonal assessment of human breast cancer, and in the identification of environmental factors and of high risk groups. However, it has been strongly felt that familial patterns and genetic influences not only relate to these problems but may be at their very core. Therefore it was decided that the main direction of the Breast Cancer Epidemiology Program this year should be toward the implementation of studies in the genetics of breast cancer.

To fulfill this objective a workshop was organized and held on September [illegible] and 11, 1975. The purpose of this [illegible] was to assemble a group of specialists in various disciplines to develop specific research protocols for genetic and pathophysiologic analysis of familial breast cancer concentrations and breast cancer differences attributed to race. In addition, conference members were asked to develop estimates of the number and character of the breast cancer lineages which would be needed for the studies, to explore the extent of the relationship inquiry needed to allow recognition and differentiation of various inheritance mechanisms, and to indicate the structure and selection of control lineages and the availability of methodology for special requisite laboratory determinations.

It was decided that these family and genetic groups should be probed for genetic marker distributions and anthropomorphic constitution, endocrinological, virological and immunological characteristics; mammary gland developmental, structural, functional and cytological features and a precise characterization of the spectrum of mammary neoplasms observed within them.

In the previous year the Committee had made a small start in the direction of family and pedigree studies by initiating a population based study in Iceland to investigate hereditary factors which might identify a high risk breast cancer-prone group. This study looks very promising and is already producing some very interesting preliminary data. In addition, the Committee had acted to support a resource of some 80 breast cancer-prone families. These had been available to collaborating investigators for a variety of clinical and laboratory studies and could serve as a nucleus for the proposed genetic studies.

To expand the genetic effort further, two RFP's were issued this year. One was aimed at soliciting proposals to do biochemical and physiological investigations based on familial genetic patterns. The objectives in this study were to compare blood and urine hormonal levels of a wide range of steroid and peptide reproductive hormones from premenopausal females of high risk breast cancer families, with those of female members of low risk families. This was an attempt to see correlations between a presumptive familial susceptibility to breast cancer and some endocrine parameters.

Studies of other physiological markers in breast cancer such as the aryl hydrocarbon hydroxylases were also included in this RFP. Eight proposals were received in response, and two will be funded.

Seven proposals were received in response to another RFP designed to investigate histocompatibility antigens (HL-A) and other genetic polymorphisms such as the immunoglobulins (Gm, Inv and A_2m), red cell enzyme (PGM), blood groups (ABO, Rh, K, Fy, Jk,MNS) and serum markers (haptoglobins, and phosphatases) in addition to chromosomal studies of selected individuals in high and low risk breast cancer families. Three contracts will be funded in this category.

The third RFP issued this year in the Epidemiology program was aimed at initiating studies to seek correlations between morphologic and epidemiologic characteristics of human breast cancers. This RFP had resulted in no awards last year. This year thirteen responses were received and three projects will be funded. In addition, an unsolicited proposal concerned with a unique morphologic classification system for breast cancer, utilizing histochemical, autoradiographic and ultramicroscopic techniques, was approved by the Committee.

An effort will also be made this coming year to organize a study in Hawaii using the unique population resource available there for a variety of epidemiologic investigations.

If the plans under way work out as expected the Breast Cancer Epidemiology program will account for approximately 10.7% of the 7-million dollar Task Force budget for FY-1974, as compared with 5.3% of the $7.2 million expended in FY-1973.

BREAST CANCER EXPERIMENTAL BIOLOGY PROGRAM

The program of the Committee continues to have as its goal "increasing knowledge of mammary tumor biology." Seven areas of investigation have been identified as of major importance in which to seek and fund projects which will contribute to that goal.

1. In Vitro Cultivation of Mammary Cells: The problem considered of major importance in this area was the long-term culture of human mammary epithelium, normal and abnormal. Three contractors have been conducting studies on this problem, one using organ culture and the others employing tissue culture techniques. An interesting and potentially important diagnostic finding in the organ culture project has been made using scanning electron microscopy: microvilli have been found on the ductal epithelium of infiltrating ductal carcinomas. These microvilli, which appear in clumps of varying lengths, have been seen in pictures of the cells prior to culturing as well as during cultivation. The investigator will concentrate his efforts on establishing the validity of these findings in the coming year.

2. Characterization of Mammary Epithelial Cells: Two of four contracts have had the objective of assessing the biological characteristics of mammary cells; one of studying migration patterns of cell types forming a monolayer from an explant in the culture media; and one of studying structural and morphological patterns of cells maintained on the chorionic allantoic membrane and in meniscus gradient cultures. Both of these contracts will be terminated in the next fiscal year. The other two contracts, initiated in June 1973, have the objective of determining biochemical and immunological cell markers for characterization of normal, preneoplastic and neoplastic mammary tissue.

3. Pathophysiology of Mammary Tumors: This area of investigation has included studies of preneoplastic and neoplastic animal and human mammary tissue. Two contracts concerning precancerous lesions have progressed to the point of being terminated. One, in which studies were conducted on morphological and functional characteristics of the human breast, will be completed in June 1974. A detailed manuscript has been submitted to the Journal of the National Cancer Institute, and other papers are planned. The other project dealt with the pathological history of the mammary gland in the beagle, and will be completed in October 1974. Some results have been published previously.

A third project has as its primary objective studies of the thymusless-nude mouse as a transplant recipient for studies on the growth potential and hormone responsiveness of human mammary tumors. Approximately 30 human breast tumor specimens have been implanted into female and male mice. A number of tumors have developed in these animals after varying periods of time and have been found to be morphologically similar to the respective primary tumors. In addition, three human breast cancer cell lines have been implanted into nude mice; one line grew rapidly in the females but failed to show growth in the males. The project will be continued, with some effort directed toward determining the effect of drugs on these tumors.

Two requests for proposals (RFP's) were issued during the past year with objectives in this area of investigation: (1) Role of stroma in the growth of neoplastic and preneoplastic lesions of the mammary gland; (2) Neoplastic potential of "preneoplastic" lesions of human mammary gland. Nine proposals were received on the first RFP and four on the second. One contract will be awarded for each RFP before July 1, 1974.

4. Hormone Responsiveness: This area of investigation is of major importance to the biology of breast cancer and therefore has received a very high priority in planning by the Committee. Eight contract projects in this area have been funded in the past two years and two others will be initiated before July 1, 1974.

One project includes studies of the role of hormones in mammary tumor pathogenesis, utilizing preneoplastic tissue from the rat. The results have shown that lesions from these glands do not progress to neoplasia as has been shown for similar lesions of the mouse. This contract is being continued.

Another important problem of hormone responsiveness is the role of prolactin in growth of the mammary tumors. Two projects on this subject are funded, one on the isolation of mammotrophs from human and rat adenohypophysis, the other on specific prolactin receptors in mammary tumors. Mammotrophs from both rat and human pituitaries have been separated and the cells have been found to retain their ultrastructural integrity and prolactin content in vitro. In the coming year co-cultivation of human mammotrophs and mammary tumor cells will be undertaken in order to determine whether the presence of mammary cells affect prolactin synthesis or release, whether the secreted prolactin is bound to mammary cells, etc. In the second project human prolactin has been produced and purified up to 70 percent, and a prolactin radioimmunoassay has been developed. With this technique prolactin levels have been measured in breast cancer patients prior to and following therapy, along with suitable normal controls. It has been found that the breast cancer patient has plasma levels of prolactin which do not differ significantly from those of normal controls. In collaboration with another contractor whose work will be reported below, estrogen binding studies have been carried out on tumors of some of these patients. Prolactin receptors have been demonstrated in DMBA-induced rat tumors for the first time. In the coming year attempts will be made to isolate prolactin receptors from cell membranes, and analyses will be made of prolactin binding fractions. There will be continuing studies of prolactin levels in breast cancer patients.

Reference was made above to studies of estrogen binding in breast cancers. Two contract investigators doing such studies are funded, one working entirely with rat tumors, the other with both human and rat neoplastic tissues. The latter is concentrating on the characterization and quantitation of estrogen binding protein (EBP) while the former is assessing the role of EBP in the growth potential of mammary tumors. An attempt to establish a correlation between estrogen receptors and objective remission is being made in the human tissue study. Considerable improvement has been made in methodology by this investigator, and the work scope has been extended to determination of prolactin and androgen receptors. The second contractor, who is studying EBP of rat cell cytosol to determine whether there is a defect of the 5S estrophile complex, has isolated the salt-stable 4.5S form of receptor protein and differentiated it from the 5S form. He has found also that: (1) nuclei of hormone-independent tumors are capable of binding estrogen to receptors, as are the nuclei of hormone-dependent ones; (2) estrogen receptor specifically stimulates RNA polymerase in target tissue; (3) an antiestrogen, Parke-Davis CI-628, has been found to produce regression of DMBA-induced mammary tumors to the same extent as ovariectomy does, approximately 80 percent. A manuscript has been submitted on these results. In the coming year tumor regression studies on the role of antiestrogens and interference with RNA polymerase activity will be carried out.

Another contract in this area relates to the role of the nucleus in hormone responsiveness. Acidic protein from the isolated chromatin of mammary gland and tumors were used for acetylation, phosphorylation or methylation, to determine whether an artificially induced defect may change the template activity of reconstituted chromatin. A technique has been developed for rapid large scale preparation of nuclei and a procedure for purification and fractionation of acidic and neutral chromosomal proteins is almost ready for publication. With hormones 90-95 percent ^{32}P binds to acidic chromosomal protein and ^{32}P is incorporated in F_{2a2} histone fraction, only during the S phase of growth. A partial fractionation of phosphorylated acidic chromosomal proteins has been accomplished. During the coming year the studies will concentrate on mouse mammary tumors, utilizing the techniques and approaches developed in the past year.

Two additional contract projects on hormone responsiveness were funded in June 1973, one to determine the response of embryonic mammary gland to androgenic hormones, the other to study the effects of Tfm mutation on the hypothalamic-pituitary axis. Both laboratories are employing pseudohermaphroditic mice. A project advertised during the past year had the title "Mammary gland responsiveness to multiple hormones". Seventeen proposals were received, two of which will be funded before July 1, 1974.

5. Mammary Tumor Growth: Last year in this report studies of growth and regression were considered together, but these are now in separate categories. During the past year four contracts were funded initially, two for studies of cyclic AMP and two on the role of prostaglandins. In the field of cAMP one investigator is determining the role of cAMP in the developing mammary gland and tumors of mice, while the other is studying possible correlation between growth rate of mammary tumors and levels of cAMP, adenylate cyclase and phosphodiesterase. The prostaglandin projects are concerned with biosynthesis in developing adult and neoplastic mammary tissue, and levels in culture media and serum from patients.

Another contract in this area of investigation concerns the role of serum factors in initiation and control of DNA synthesis in cultures of mouse mammary epithelium. A fraction has been obtained from serum which has a growth-promoting effect on mammary cells 44 times greater than that of normal serum. In the coming year this factor will be purified further and an antibody to it will be prepared. In studies to date it appears to be similar in action to somatomedin. Other results have shown that insulin is not the growth-promoting factor of whole serum.

6. Mammary Tumor Regression: Two new projects were advertised in this area of investigation: (1) Transfer of nucleic acids into mammary cells and possible changes of their growth potential; (2) Growth alteration of mammary tumor obtained by manipulation of the cellular environment. Fifteen proposals were received for the first-named and five for the second. One contract has been selected for each, to be funded before July 1, 1974.

Two of the projects concerning mammary tumor regression will be terminated: a project on the antitumor effect of methanol-insoluble residue of BCG which will be completed in June 1974; and one on involution of the mammary gland *in vivo* compared with loss of differentiation of lactating mammary gland when transferred *in vitro*.

Two projects in this field were initiated in June 1973, one on interactions between lymphocytes and mammary cells, normal and abnormal, the other on the inhibition of cellular immunity by mammary tumor cells.

7. Metastasis: One of five contract projects in this area of investigation is evaluating the metastasizing capacity of tumor *per se*. The other four are supportive to the type of studies ongoing in the first contract, as well as to projects described under the other areas of investigation. One of the four, concerned with changes during differentiation or oncogenesis, will be terminated during the coming year.

In the project on the metastasizing capacity of tumors, rat mammary carcinomas have been employed. A number of spontaneously metastasizing tumors have been isolated and studied for differences in membrane properties. Ascites cell lines have been developed from most of the metastasizing and non-metastasizing neoplasms, and most of these can be maintained in monolayer culture. These tumors will be studied under this project as well as others funded through this Committee. Non-metastasizing tumors have several hundred times more activity of alkaline phosphatase, 5'-nucleotidase, phosphodiesterase, nucleotide pyrophosphotase, and aklaline ribonuclease, than metastasizing ones. Cyclic AMP levels were about twice as high in metastasizing tumors. Studies of the lysosomal enzymes and cell surface antigens of the two types of tumors will be carried out in the coming year.

Two contracts were initiated in June 1973 which will contribute data for the above program and others: one for the development of techniques for the isolation of epithelial cell membranes; and one for the study of the composition and biochemical properties of mammary cell plasma membranes.

The fifth contract in this area is service in nature and provides a tumor bank for storing and characterizing mammary and pituitary tumors. Specimens of these are sent to other contract investigators in the Biology program and on a limited basis to outside investigators.

Summary: At the beginning of Fiscal Year 1975 the Experimental Biology Committee will have a total of [illegible] contracts — [illegible] renewals and 6 new ones. Five of the renewal contracts will be completing their terminal year during Fiscal '75. Two contracts will have been terminated in June 1974 and one will be terminated in October 1974. In the past fiscal year five subjects were advertised in the Commerce Business Daily and the NIH Guide to Grants and Contracts. Fifty proposals were submitted in response to the advertisements and six were selected by the Committee for funding.

Present indications are that the Breast Cancer Experimental Biology program will use approximately [illegible]% of the 7-million dollar budget for FY-1975, as compared with 40.0% of the $5.2 million expended in FY-1974.

BREAST CANCER TREATMENT PROGRAM

The principal developments in the Treatment program during the last year emphasized three main areas: (1) in therapy, evaluation of the relative effectiveness of current methods, particularly with the view of introducing effective adjuvant treatment after mastectomy; (2) in cytokinetics, a search for new methods of measuring growth potential and of identifying, in the individual patient, phases of cancer progression most susceptible to treatment, and (3) in the field of "markers" to check out some current ideas for identifying substances in blood or tumor that would give a measure of tumor load, early presence of tumor or characteristics of the host or tumor having prognostic significance.

In the evaluation of therapy, the National Surgical Adjuvant Breast Project has almost finished entering patients in the study comparing total and radical mastectomy, and is approaching significant findings in the study of L-phenylanine mustard as an adjuvant to surgery of primary breast cancer. Protocols are being prepared for further studies in surgery and adjuvant therapy, their exact form being dependent on the conclusions of the parent studies. A separate study is evaluating surgical adjuvant chemotherapy -- 5FU, cytoxan and prednisone with or without postoperative irradiation -- in patients undergoing modified radical mastectomy. Finally, during the current year, two new RFP's were published, one for studies on "Hormonal Manipulation plus Chemotherapy in Patients with Breast Cancer", and for "Therapy of Patients Stage II and III Carcinoma of the Breast". The latter called for the use of patients treated with concurrently or sequentially combined modalities which could include surgery, radiotherapy, chemotherapy, hormonal manipulation and immunotherapy. Responses to these RFP's have been reviewed, and it is anticipated that proposals will be funded totalling $300 - 350,000 for each RFP.

In the field of cell kinetics, two advertisements had been issued in FY-1974. One called for the development of an experimental approach to the study of tumor growth kinetics "potentially applicable to the study of patients with breast cancer". The other called for the study of cell kinetics in patients with breast cancer, including cases before and after endocrine-ablative therapy, hormones, or chemotherapy. Four projects, two each responsive to these RFP's got under way in the beginning of the current period, and are progressing satisfactorily.

The term "markers" includes substances and reactions scientifically diverse but potentially of great practical value in diagnosing or monitoring disease or the state of the patient. Thus, measurement of plasma prolactin represents an attempt to determine how this hormone relates to breast cancer in patients while as yet it is largely animal experimentation that suggests a strong etiological connection. In the treatment program a core laboratory has been set up for exploratory collaborative studies on prolactin in patients with breast cancer or control subjects of different risk categories. Some theoreti speculations by Dr. Thomas Dao led to the clinical observation that regression after adrenalectomy occurred most frequently in patients whose tumors had enzymes that sulfurylated dehydroepiandrosterone more efficiently than estradiol. The Breast Cancer Treatment Committee felt that this work was sufficiently promising to justify its continuation and expansion as an unsolicited contract project.

The best known and probably most promising "marker" is one that presumably distinguishes between tumors which are endocrine-responsive and those which are not. This marker came to light through the research of Dr. Elwood Jensen and others on the estrogen-binding proteins of normal breast and uterine tissue. Six years ago Dr. Jensen suggested a possible correlation between the estrogen-receptor level of breast tumors and their liklihood of responding to therapies, like adrenalectomy, aimed at the removal of sources of estrogen. The Task Force supported this line of research, and considerable data have been accumulated by Jensen and others indicating that the correlation may exist. The Breast Cancer Treatment Committee has moved to extend and clarify the potential use of this marker in three ways: it is supporting extended methodological and clinical studies by Dr. Jensen's group; it has set up a central laboratory to carry out "blind" collaborative studies on estrogen receptor under a variety of controlled clinical conditions; and it is sponsoring a Workshop on Estrogen-Receptor Clinical Correlations to bring together all the investigators who have data on this subject. Estrogen receptor as a marker may be only a beginning; the long range aim is to define "hormone dependency" in terms of a spectrum of receptor materials.

The Treatment program in FY-1973 received approximately 25.3% of the $5.2 million used in the BCTF program; in FY-1974 its share is expected to be about 24% of a $7.0 million program.

CONFERENCES AND WORKSHOPS

From time to time the Task Force has found it useful to hold a workshop to review progress in the whole or part of the program or to clarify the state of the art in one of its areas. Typical of the latter were two workshops sponsored by the Epidemiology Committee to determine how recent developments in endocrinology and genetics should relate to the Epidemiology program. Typical of the former were the comprehensive reviews of the treatment of breast cancer, sponsored by the Treatment Committee, and the two annual program reviews sponsored by the Steering Committee.

The annual review conference sponsored by the Steering Committee has become a necessary annual event, bringing together contractors, members of advisory groups, guests and NCI staff for intensive discussion of the program. The first was held in February 1973 and was attended by 111 participants; the second, in February 1974, was attended by [illegible]. The third conference of this type will be held in February 1975, at a site not yet selected.

The following is a list of working conferences held under various BCTF auspices since January 1972, and those scheduled through February 1975. Two or three others, not listed, are in the early planning stage.

January 31, 1972 and February 1, 1972	"Current Clinical Research in the Treatment of Breast Cancer" (Bethesda). Sponsored by the Breast Cancer Treatment Committee.
June 26-27, 1972	"Workshop on Breast Cancer" (Bethesda). Sponsored by the Epidemiology Committee.
August 1-4, 1972	"Workshop on Culture of Human Mammary Tissue" (pub. in JNCI 50 (5): 1407-1410, May, 1973). Asilomar Conference Grounds, Pacific Grove, California.
February 5-7, 1973	"First Breast Cancer Task Force Working Conference" (Williamsburg). Sponsored by the BCTF Steering Committee.
May 17-18, 1973	"Breast Cancer Treatment Meeting" (Bethesda). Sponsored by the Treatment Committee.
September 10-11, 1973	"Workshop on Genetics and Susceptibility to Human Breast Cancer" (Bethesda). Sponsored by the Epidemiology Committee.
February 4-6, 1974	"Annual Progress Review Conference, Breast Cancer Task Force" (Denver). Sponsored by the BCTF Steering Committee.
July 18-19, 1974	"Workshop on Estrogen Receptor Clinical Correlations". Sponsored by the Treatment Committee.

FUTURE PROGRESS

Most of the major fields considered important to progress against breast cancer by the Task Force have now received some attention and exploration. Time will probably reveal some blind spots and also focus attention on areas that have been relatively neglected. Meanwhile it is anticipated that some long-term studies begun earlier will begin to yield information, at least enough to point to further tasks that need to be undertaken. Special emphasis this coming year will be placed upon interaction between committees of the Task Force and between the Task Force program and other programs dealing with breast cancer -- especially those of the Medical Oncology Branch (DCT), Viral Oncology, Biometry and Epidemiology Branches (DCCP), and the Treatment Branch in Cancer Control. Common interests among these groups have already led to some joint activities in the field of breast cancer, and can be expected to lead to others.

CONTRACT RESEARCH SUMMARY

Title: Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer

Principal Investigator: Evan C. Horning, M.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas
Organization:

Contract Number: N01-CB-43897
Starting Date: January 1, 1974 Expiration Date: 12/31/74
Goal: To make a detailed comparison of steroid metabolites in urine and blood in a search for a possible diagnostic marker for breast cancer.

Approach: Urinary free and conjugated steroid metabolic profiles will be determined using gas chromatographic - mass spectrometric techniques on a group of 25 patients with breast cancer and compared with similar profiles for normal controls and for patients with benign lesions. These studies will be carried out with two 24-hour urine collections for breast cancer patients, for patients with benign lesions and for matched controls. The postmenopausal age group (50-60) will be studied in the first year, the premenopausal group in the second year. Another hypothesis to be tested is that breast cancer is caused by steroidal epoxides formed in breast tissue or through liver metabolism. This proposed work involves a study of blood and urine of breast cancer patients for trace amounts of steroidal epoxides, and for possibl precursors and metabolic products by means of gas phase analytical methods.

Progress: New Contract - Semi-annual progress report due July 1, 1974

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 11/19/1973
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $116,200

CONTRACT RESEARCH SUMMARY

Title: Development of Immunological Assays for Human Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. Anthony Monaco Boston City Hospital (Harvard University) Boston, Massachusetts

Contract Number: N01-CB-23870

Starting Date: June 20, 1972 Expiration Date:

Goal: To perform a series of assays of cell mediated immunity in patients with breast cancer and to determine their usefulness in prognosis and in detecting residual disease.

Approach: Patients with breast cancer are being tested for reactivity in several assays of cell-mediated immunity: skin reactivity to a standard battery of recall antigens and to extracts of breast tumor cells, sensitization to dinitrochlorobenzene, cell-mediated cytoxicity against tissue culture cells derived from breast tumors, lymphocyte stimulation by tumor cell extracts, and inhibition of leukocyte migration by tumor cell extracts. These results are being correlated with the clinical stage of disease and response to therapy.

Progress: All the above assays have now been set up and data is being accumulated. Many of the technical problems associated with these assays have been worked out. Twelve of nineteen patients admitted to the study since August, 1972 are being followed by periodic clinical examinations and immune assays. Thus far, 137 skin tests have been performed with non-specific antigens and specific breast tumor antigens. Ten extensive lymphocyte transformation studies have been performed. Four patients have been studied with M.I.F. (migration inhibition factor) assay and their own breast tumor antigen. Forty-five in vitro microcytotoxicity assays for C.M.I. (cell-mediated immunity) and serum blocking factors (S.B.F.) have been done.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Assays for cell-mediated immunity can be potentially useful for diagnosis and prognosis.

Project Officer: Ronald B. Herberman, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 3/1/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: none

CONTRACT RESEARCH SUMMARY

Title: Analysis of Breast Secretions

Principal Investigator: Name/Address Performing Organization:	Dr. N. L. Petrakis University of California San Francisco, California

Contract Number: NO1-CB-33882

Starting Date: May 15, 1973 Expiration Date: 5/14/75

Goal: To evaluate Sartorius breast pump technique as a means of breast cancer detection, using cytological, biochemical and virological analysis of secreted material.

Approach: Obtain breast secretions by means of a pump developed by Dr. O. Sartorius. Secretions will be analyzed by cytological, virological and biochemical methods, and correlated to mammographic and pathological findings. In select cases breast intraductal catheterization will be carried out to pinpoint locations of suspected lesions.

Progress: Nipple aspiration was attempted in 604 normal women of the major racial groups. Studies were made to describe the non-neoplastic cellular composition of fluid aspirates. By February 1, 1974, 91 women who had suspected lesions based on clinical and/or mammographic findings were subjected to a nipple aspiration. The cytologic findings were correlated with mammographic and pathologic findings. There is not enough data at present to make meaningful correlations; however they offer encouragement that this technique may clinically complement or supplement mammography as a preoperative diagnostic tool.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Extend the cytologic and biochemical detection methods.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/25/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $184,600

CONTRACT RESEARCH SUMMARY

Title: Purification of an Antigen from Human Mammary Carcinoma

Principal Investigator: Name/Address Performing Organization:	Dr. Donald Marcus Albert Einstein College of Medicine Bronx, New York

Contract Number: NO1-CB-23871
Starting Date: June 20, 1972 Expiration Date: 6/19/75

Goal: To identify antigens associated with human breast cancer and to develop a quantitative assay for this antigen, for potential use in the immunodiagnosis of breast cancer.

Approach: The contractor's approach is to develop antisera specific for the antigen(s) and to purify the antigen(s). Tests for specificity will be performed with breast tumor tissues, normal breast tissues, and other control tissues. Contaminating antibodies or antigens will be removed by immunoadsorbents and by fractionation procedures. Isolated and purified antigen will be labelled with ^{125}I and used in a double antibody radioimmunoassay.

Progress: During the first contract year, the contractor produced an antiserum which reacts with extracts of primary and metastatic breast cancer and does not react with similar extracts of normal breast tissue. A single band is seen on immunodiffusion. This antiserum has been used in affinity column chromatography for isolation of the antigen. In the past year the antigen detected earlier has been purified and identified as ferritin. A double-antibody radioimmunoassay for ferritin in human serum has been developed. Of the 650 sera obtained from 568 patients, of whom 30% have breast cancer, 30 sera have been assayed for serum ferritin to establish normal limits. The sera of 96 patients with documented breast cancer have also been assayed. Acidic ferritins from human tumors are being isolated for the study of immunological properties and to determine the chemical basis of their electrophoretic differences from normal ferritin.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Immunodiagnostic assays offer the potential for early diagnosis of breast cancer.

Project Officer: K. Robert McIntire, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1/18/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $85,008

CONTRACT RESEARCH SUMMARY

Title: Ultrasound Mammography

Principal Investigator: Dr. Gilbert Baum
Name/Address Performing Organization: Albert Einstein College of Medicine
Bronx, Nes York

Contract Number: N01-CB-23872
Starting Date: June 20, 1972 Expiration Date: 6/18/75
Goal: To develop ultrasonic diagnostic criteria for malignant breast lesions.

Approach: Ultrasonic "viewing" has had highly successful application for diagnostic purposes in some organs. The contractor is world's authority on the use of ultrasonics in eye examinations. The need for nonradiational detectors of breast tumors is very high. The contractor's aim was to develop a new scanner suitable for breast work, and to lay foundation for ultrasonic mammography surveys.

Progress: Preliminary designs of the new scanner have been experimented with. Actual construction was hampered by lack of suitable commercially produced units, making the timetable some 3-6 months behind schedule. Breast examinations were carried out on prototype eye instruments. Ultrasonographic diagnostic criteria for identifying malignant change in breast and most common diseases of breast have been developed. The range of "normal" ultrasonographic anatomic breast patterns has been established. The investigators have designed and begun building a breast scanner, sweep circuits and STC circuits for ultrasound mammography.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A new modality for diagnostic purposes.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 11/16/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $172,936

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Patient Input-Follow-up

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Robert W. Talley
Henry Ford Hospital
Detroit, Michigan

Contract Number: N01-CB-23230
Starting Date: April 3, 1972 **Expiration Date:** 4/2/75
Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: About 30 patients were to be entered by the contractor in the first contract year. This year's contract is devoted to the follow-up of approximately twenty patients at three-month intervals.

Significance for Cancer Research (NCP Objective 6 Approach 6)
Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis **Site Visit Date:** 3/2/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $8,670

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator Name/Address Performing Organization:
Dr. Etienne Lasfargues
Institute for Medical Research
Camden, New Jersey

Contract Number: NO1-CB-[illegible]
Starting Date: June 1, 1972 **Expiration Date:** 5/31/75

Goal: To develop techniques for more reliable growing tumor cells and normal control cells from breast cancer patients in tissue culture, and to supply these cells for use in immunodiagnostic assays.

Approach: Tumor cells and normal breast tissue cells are obtained and placed into tissue culture. A variety of procedures for harvesting the cells will be attempted and various media and supplements will be tested for their ability to support cell viability and growth. Primary culture cells and established cell lines will be frozen in viable condition and either fresh or frozen cells will be made available to other investigators in the program for use in immunodiagnostic assays.

Progress: During the first contract year the logistics for obtaining tissue specimens from local sources and from other NCI contractors were worked out. About 50% of the tumor cells received were successfully maintained in culture for short periods of time. In the next year the following goals were achieved: (1) the isolation of viable epithelial cells from 75% of scirrhous carcinomas, (2) the elimination of fibroblasts from cultures, (3) the stimulation of mitotic activity with mitogenic factors liberated by embryonic cells, (4) the finding of active cell-mediated factors for the establishment of breast tumor lines that have been growing for several generations, many of which have been sent on request to 22 investigators and (5) the establishment of cell lines from solid breast tumors.

Significance for Cancer Research (NCI Objective 5 Approach 4)
Tissue cultures of tumor cells are essential for development of cell-mediated cytotoxicity assays, which have potential usefulness in the immunodiagnosis of cancer.

Project Officer: Dr. Chou-Chik Ting
Program: Breast Cancer Diagnosis **Site Visit Date:** 2/28/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $67,000

CONTRACT RESEARCH SUMMARY

Title: Experimental Design and Statistical Services for Studies in the Treatment of Primary Breast Cancer

Principal Investigator: C.A. McMahan, Ph.D.
Name/Address Louisiana State University
Performing New Orleans, Louisiana
Organization:

Contract Number: N01-CB-50573
Starting Date: February 15, 1965 **Expiration Date:** 3/31/75

Goal: To provide statistical management, consultation and services for studies of recurrence and survival in primary female breast cancer being carried out by certain members of the Cooperative Breast Cancer Study Group.

Approach: The principal investigator participates in the planning of studies to be carried out and manages the collection, analysis, and evaluation of data pertaining to preliminary studies of accuracy and reproducibility of methods and to major studies which are carried on by the Study Group. He and his staff design forms for data collection, supervise the labeling and handling of specimens for laboratory analysis on a blind basis, and collect and analyze the resulting data. Laboratory and clinical data are reduced to punch cards or computer tape for tabulation and analysis. Reports are prepared for the guidance of the investigators.

Progress: This contractor has demonstrated capability in the operation of a statistical center for this collaborative study of relating clinical, pathologic and urinary steroid factors to risk of recurrence in breast cancer. He has successfully standardized procedures for both the urinary steroid laboratory and the pathology laboratory so that reproducible results are obtained. He will continue efforts to evaluate additional predictor variables and to explore methods for improving "predictive" (classification) functions. He wil continue to apply quality control procedures to data processing data collectio and data reporting as well as critical review of data already gathered.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Project Officer: Max H. Myers, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 10/17/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Dr. Anne C. Carter
Name/Address Downstate Medical Center (SUNY)
Performing Brooklyn, New York
Organization:

Contract Number: N01-CB-23231
Starting Date: April 16, 1972 Expiration Date: 4/14/75
Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: About 70 patients were to be entered by the contractor in the first contract year. After patient accrual had terminated, a follow-up period of two years was instituted.

Significance for Cancer Research (NCP Objective 6 Approach 6)
Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 6/8/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $25,000

CONTRACT RESEARCH SUMMARY

Title: Mass Screening for Breast Cancer Electronic Infrared Pattern Recognition

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address
Performing
Organization: University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma

Contract Number: N01-CB-43869
Starting Date: April 1, 1974 Expiration Date: 9/30/75

Goal: To design and clinically test a prototype system for mass thermographic screening of breast cancer and couple it with computer processing for automated evaluation.

Approach: This project is phase I of a planned 3-phase development program. The first phase would include: (1) construction of an improved instrument utilizing the unique serial scan multiple element developed by Honeywell. The instrument will be equipped for direct recording on a standard video recorder and conventional television display, (2) a program of clinical evaluation to assess the utility of the various features in the diagnosis of breast cancer and compare with results of other modalities, (3) definition of the system specifications for a mass screening prototype instrument, and (4) development of initial data base for automated screening evaluation.

Progress: Honeywell is currently beginning assembly of an infrared scanner using a multiple element mercury cadmium teluride detector. As soon as the instrument is available the clinical portion of the project will be conducted in the University of Oklahoma Breast Cancer Screening Center.

Significance for Cancer Research (NCP Objective 5 Approach 3)
This approach would extend and improve procedures for mass screening of population groups.

Project Officer: Kenneth Olson, M.D. and William Pomerance, M.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/12/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $234,000

CONTRACT RESEARCH SUMMARY

Title: Electronic Mammography

Principal Investigator: Dr. Donald Sashin
Name/Address University of Pittsburgh
Performing Pittsburgh, Pennsylvania
Organization:

Contract Number: N01-CB-33904
Starting Date: June 30, 1973 Expiration Date: 12/29/74
Goal: Develop electronic radiography technique designed for mass screening for early breast cancer detection.

Approach: Electronic radiography involves substitution of image intensification and TV techniques for X-ray film, increasing the efficiency with which X-rays can be recorded, thus reducing the X-ray dose. The program consists of two parts: (1) design and construction of a prototype system optimized for visualization of soft tissues and (2) evaluation of the system regarding its ability to display clinical details relative to present mammography system.

Progress: During the first period of the contract measurements have been made of the total system performance with the intention of improving the image quality to visualize small pathology. Extensive engineering, computer and phantom evaluations have been carried out to optimize the system performance.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 2/27/74
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: Six months extension with no additional funds

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients with Breast Cancer*

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Per Vestergaard
Research Foundation for Mental Hygiene
Orangeburg, New York

Contract Number: N01-CB-71465
Starting Date: June 28, 1967 Expiration Date: 6/27/74*
Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross-contamination.

*After 6/27/74, contract is transferred to National Heart Lung Institute, with Dr. Frederic C. Bartter as Project Officer.

Progress:

Significance for Cancer Research (NCP Objective 6 Approach 6)
This contact represents one of our two efforts in the area of steroid analysis, serving several clinical studies.

Project Officer: Dr. Frederic C. Bartter
Program: Breast Cancer Diagnosis Site Visit Date: 3/28/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $55,700 (NHLI funds)

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator: **Gilbert Friedell, M.D.**
Name/Address **St. Vincent Hospital**
Performing Worcester, Massachusetts
Organization:

Contract Number: N01-CB-80704
Starting Date: March 18, 1968 Expiration Date: 4/17/75
Goal: To continue the operation of a central pathology laboratory as part **of the Primary Therapy of Breast Cancer Study.**

Approach: The objective of the above study is to find a correlation between clinical, pathologic and urinary steroid findings which would serve as prognostic indices in women with breast cancer. The central pathology laboratory was established for this study to achieve a standardization of sampling and preparative procedures for histopathologic studies and uniform systematic evaluation. Clinical material is being provided by the members of the **Cooperative Breast Cancer Group.**

Progress: The initial pathologic studies aimed at assessing the presence of metastases in regional lymph nodes, the presence of blood cell invasion and nuclear grade at the primary tumor site. The data is now being analyzed for correlation with clinical status and urinary steroid findings.

Significance for Cancer Research (NCP Objective____ Approach____)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: No cost extension

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Tissue Supply

Principal Investigator: Dr. Paul O'Brien
Name/Address
Performing
Organization: Medical College of South Carolina
Charleston, South Carolina

Contract Number: N01-CB-23867
Starting Date: June 30, 1972 Expiration Date: 6/29/75

Goal: To obtain tissue and blood specimens from patients with breast cancer for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before surgical therapy and at regular intervals and thereafter. These specimens are shipped promptly to other investigators in the program for use in immunodiagnostic assays.

Progress: During the first contract year, the contractor has supplied more than the minimum number of specimens specified. The logistics for obtaining specimens and for satisfactory shipment have been worked out successfully. From June, 1973 to January 1, 1974, 96 blood specimens, 46 malignant tumors (with some normal tissue) and 19 benign tumors have been sent. From October 1, 1973 to January 1, 1974, 29 follow-up blood specimens were also shipped. Currently follow-up blood specimens on all study patients with malignant tumors are being shipped on a weekly and monthly basis. The University has been 95% successful in obtaining follow-up blood specimens on study patients.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Specimens from breast cancer patients are needed for rapid development of potential immunodiagnostic assays.

Project Officer: Dr. Ronald B. Herberman
Program: Breast Cancer Diagnosis Site Visit Date: 5/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $75,500

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator: William Reeves and Relda Cailleau
Name/Address M. D. Anderson Hospital
Performing (University of Texas)
Organization: Houston, Texas

Contract Number: NO1-CB-23869
Starting Date: June 1, 1972 Expiration Date: 8/1/74
Goal: To develop techniques for more reliably growing tumor cells and normal control cells from breast cancer patients in tissue culture, and to supply these cells for use in immunodiagnostic assays.
Approach: Tumor cells and normal breast tissue cells are obtained and placed into tissue culture. A variety of procedures for harvesting the cells will be attempted and various media and supplements will be tested for their viability and growth. Primary culture cells and established cell lines will be frozen in viable condition and either fresh or frozen cells will be made available to other investigators in the program for use in immunodiagnostic assays.

Progress: Complete tissue culture facilities have been set up and the logistics for obtaining tissue specimens and for shipping specimens and culture cells have been worked out. Cells from both primary breast tumors and from malignant pleural effusions have been placed into culture. In the past year temporary cell growth has been achieved in 30 to 50% of 53 solid breast tumors cultured but no breast tumor lines were established. However 3 epithelial cell lines have been established from 42 metastatic pleural effusion specimens. In a collaborative project, aliquots of the same materials have been sent to Dr. Lasfargues and to Dr. Grace Cannon. This has allowed comparison in culture techniques and in interpretation of cell outgrowths. Most of the biopsy cells and the cells in culture have been examined carefully by electron microscopy for tumor cell verification.

Significance for Cancer Research (NCI Objective_5_Approach_4_)
Tissue cultures of tumor cells are essential for development of cell-mediated cytotoxicity assays, which have potential usefulness in the immunodiagnosis of cancer.
Project Officer: Dr. Ronald B. Herberman
Program: Breast Cancer Diagnosis Site Visit Date: 5/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $133,800

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs Film Mammography - A Comparative Study

Principal Investigator:

Name/Address Performing Organization:	Gerald Dodd, M.D. M.D. Anderson Hospital, Houston TX	Richard Lester, M.D. Duke University Durham, NC	Philip Strax, M.D. Guttman Institute New York, NY

Contract Number: New Contract

Starting Date: **Expiration Date:**

Goal: To evaluate the relative merit of conventional radiography vs xeromammography in the detection of breast cancer and to delineate differences which may suggest the use of one method over the other in certain situations.

Approach: This project will be developed among three institutions, University of Texas M.D. Anderson Hospital and Tumor Institute, Duke University Medical Center, Stella and Charles Guttman Breast Diagnostic Institute. It is proposed to assess the mammographic image formation and information transmitting characteristics of each process. Such factors as radiographic contrast, latitude, sensitivity, and modulation transfer function affect the ultimate transmittal of information. Methods to assess these factors will be used to determine optimum techniques in both types of radiography.

Specifically, the Guttman staff will provide a dosimetry and calibration service to insure that the physical condition of exposure, processing and viewing are adequately known and sufficiently similar at each of the three institutions.

The Duke staff will provide spectrometric services such that the two modalities can be compared under known spectral conditions.

The Anderson staff will assess the information and transfer characteristics of the imaging system. Preliminary measurements will be carried out at the Image Research Center at the University of Chicago under sub-contract; these will include modulation transfer function for the baseline film mammographic recording medium and the screen film mammographic system manufactured by DuPont.

An advisory and monitoring body will be constituted which will render judgment upon the technical quality of work performed by each of the participating institutions.

Since the subjective evaluation of the individual examinations should not depend entirely upon the principal investigators as individual bias will intrude, the cephalocaudad films from all institutions will be reviewed by a panel, the members of which will be named by the Diagnostic Radiology Committee of the National Cancer Institute.

Progress: New Contract

Significance for Cancer Research (NCP Objective___Approach___)

Project Officer: William Pomerance, M.D.

Program: Breast Cancer Task Force

Technical Review Group: Diagnostic Radiology Committee for Breast Cancer Task Force

Relevance Review Group: Breast Cancer Steering Committee

FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Fibrocystic Disease of the Female Breast and its Relationship to Mammary Carcinoma

Principal Investigator: David L. Page, M.D.
Name/Address Vanderbilt University
Performing Nashville, Tennessee
Organization:

Contract Number: NO1-CB-43871
Starting Date: January 15, 1974 Expiration Date: 1/14/75
Goal: To determine which lesion or which combination of lesions, if any, in chronic cystic mastopathy leads to the development of mammary carcinoma.

Approach: The investigator is assembling, reviewing and classifying slides from breast biopsies of 1000 women performed 15 or more years ago and diagnosed as cystic disease at that time. All slides will be classified as to component parts of cystic disease according to the Foote and Stewart classification system. Patients will be followed until their death or the development of mammary carcinoma. Each patient, each patient's record, and physician or family will be contacted to secure the desired information. Correlative studies between the lesions of cystic disease and the development of mammary carcinoma will be undertaken. An attempt will be made to demonstrate how various kinds of cystic disease lesions are associated with the development of mammary carcinoma.

Progress: New Contract. Semi-annual progress report will be due on July 15, 1974.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $53,600

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Carleton R. Haines
University of Vermont
Burlington, Vermont

Contract Number: NO1-CB-23232
Starting Date: March 13, 1972
Expiration Date: 3/12/75
Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: Forty-six (46) patients have been entered since June 15, 1972. Twenty-six of these have received the 3 and 6 month follow-up examination. Eight patients have been followed over one year. This is the terminal year of follow-up, plus evaluation of results and preparation of reports.

Significance for Cancer Research (NCP Objective 6 Approach 6)
Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Site Visit Date: 6/9/72
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $8,200

CONTRACT RESEARCH SUMMARY

Title: Determinations of Steroid Sulfate Content in Blood to be Used as Diagnostic Parameters in Breast Cancer

Principal Investigator: Federico Welsch, M.D., Ph.D.
Name/Address Performing Organization: Worcester Foundation for Experimental Biology, Inc.
Shrewsbury, Massachusetts

Contract Number: NO1-CB-43898
Starting Date: January 1, 1974 Expiration Date: 12/31/75
Goal: To analyze blood steroid sulfate levels to serve as possible diagnostic and/or prognostic parameters in breast cancer.

Approach: Estrone sulfate, dehydroepiandrosterone sulfate and androsterone sulfate will be measured in the plasma of at least one hundred patients per group, either with diagnosed breast cancer or diagnosed benign breast tumors or apparently normal controls using radioimmunoassay techniques. These measurements can be used to indicate possible changes in adrenal steroid production in postmenopausal women and adrenal and ovarian activity in young women occurring after the development of breast cancer. It would be an attempt to find a unique metabolite in blood which might be characteristic of breast cancer.

Progress: New Contract - Semi-annual progress report will be due on July 1, 1974

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Ihor Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 9/4/73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
[illegible] Funds: $97,700

CONTRACT RESEARCH SUMMARY

Title: Automated Methods for Determination of Steroid Metabolites

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Federico Welsch
The Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Contract Number: N01-CB-60968
Starting Date: June 10, 1966 Expiration Date: 6/9/75
Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: This analysis of steroid hormones is based on paper chromatography with uniquely designed triple wavelength readout permitting and automated Allen correction to be incorporated. The methodology is highly specific and reproducible, permitting the analysis of a spectrum of hormonal metabolites on 5-10% aliquot of a 24-hour specimen.

Progress: During the year 1973-1974 the contractor exceeded the specifications for work load by 200% without additional cost. The laboratory serves as the analytical arm of the clinical study designed to develop a predictive index for spotting early recurrences after radical mastectomy. When patient accrual and follow-up ends, the contractor will be checking out one year postmastectomy values of urinary steroids.

Significance for Cancer Research (NCP Objective 6 Approach 6)
This contract represents one of our two service components to the clinical studies.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: 1971
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $50,000

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Family Resources

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Creighton University
Performing School of Medicine
Organization: Omaha, Nebraska

Contract Number: N01-CB-33901
Starting Date. June 30, 1973 Expiration Date: 6/29/75
Goal: To establish a research resource pool of about 80 well-documented breast cancer-prone families to be used for medical genetic studies.

Approach: There will be continuous updating of genealogy and tumor history by personal interviews with patients and physicians and by telephone calls and letters. An augmentation from 50 (present members) to 80 families is planned. A critical analysis of these families will be continued in a search for basic genetic mechanisms through the study of genealogy and varying combinations of malignant neoplasms as well as other medical disorders in this series of breast cancer-prone families. Pedigrees with detailed description which are compiled upon each family will be made available for review by each contractor who expresses an interest in studying these families. A mechanism for handling applications for access to the resource by qualified investigators (with separate funding) will be developed in consultation with Breast Cancer Task Force members and staff.

Progress: Approximately 85 families are now available in the breast cancer family resource population, 30 of which have been ascertained in the past year. Studies in many of these, i.e. full ascertainment of genealogy, medical and pathology documentation is still in progress. Individuals in each group range from 10 to 250. Those available in all families (most recent census) number over 2000. In the 54 breast cancer families fully ascertained there are 8 site specific breast cancers, 6 with predominance of breast, ovarian and endometrial carcinoma, 15 with breast and GI cancer and others with combinations of breast and a variety of other malignant neoplasms.

Significance for Cancer Research (NCP Objective_5_Approach_1_)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/19/73
Technical Review Group: Breast Cancer Epidemiology Committee
Reference Review Group: Breast Cancer Steering Committee
FY74 Funds: $64,424

CONTRACT RESEARCH SUMMARY

Title: Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: Henry T. Lynch, M.D.
Name/Address Performing Organization: Creighton University School of Medicine
Omaha, Nebraska

Contract Number:
Starting Date: Expiration Date:
Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high and low risk breast cancer families.

Approach: HL-A markers, genetic polymorphic markers such as red blood cell enzymes, (acid phosphatase, adenosine deaminase, etc.), serum proteins (haptoglobins α chain, group-specific component, etc.), and blood group systems (ABO, Duffy, Lewis, MN, etc.) and specific pathologic diagnoses (all histologic varieties of cancer retrospectively in the family context) will be evaluated in approximately 20 mulitple cancer case families selected from the total clinical pool of 67 high risk families and kindreds.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/5/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $94,419

CONTRACT RESEARCH SUMMARY

Title: A Study on Familiality of Breast Cancer in Iceland

Principal Investigator: Name/Address Performing Organization:	Dr. Hrafn Tulinius and Professor O. Bjarnason International Agency for Research on Cancer Lyon, France

Contract Number: NO1-CB-02076
Starting Date: June 30, 1973 Expiration Date: 6/29/75

Goal: To determine whether there are hereditary characteristics which might be of use in the identification of women having a high risk of developing breast cancer.

Approach: The unique facilities available in Iceland for the study will be utilized. These include 1) a nationwide cancer registry functioning since 1954 (about 800 cases), 2) a computer based National Roster since 1952, and 3) a family linkage file linking every individual alive after 1910 to his parents and children. If an excess risk is found, an effort will be made to 1) correlate familial risk with recognized eqidemiological risk factors for breast cancer, and 2) quantify the risk attached to different types of relationship; first, by relating degree of risk to degree of relationship, one can attempt to separate genetic from environmental factors and second, by comparing degree of risk among maternal relatives with that among paternal relatives, one can test for the extrachromosomal inheritance of mammary cancer, and 3) for each class of relative, examine the association, if any, between the degree of risk and the age of the proband case and age of appearance of cancer among these relatives.

Progress: Family linkage file is under construction and is at different levels of completion. It can be considered 100% complete for those born in or after 1916 and can identify all persons alive at the time of 1910 census. A birth record from 1840 is being reconstructed and is complete for about half the population. Analysis of incidence data from 1911-1972 has shown a marked increase in breast cancer during the period and a corresponding change in the shape of the age incidence curve. This change in shape can be explained solely as a cohort effect.

Significance for Cancer Research (NCP Objective 5 Approach 1)

A population based study is needed to determine whether genetic factors can serve to identify a high risk breast cancer-prone group.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $30,400

CONTRACT RESEARCH SUMMARY

Title: Epidemiological and Etiological Characteristics of Chronic Cystic Mastopathy and Relationship to Breast Cancer

Principal Investigator: **Name/Address** **Performing Organization:**	Dankward Kodlin, M.D., M.P.H. Kaiser Foundation Research Institute Oakland, California

Contract Number: N01-CB-33921
Starting Date: June 30, 1973 **Expiration Date:** 6/29/75

Goal: Studies will be formulated to investigate criteria for the designation of atypia in chronic cystic mastopathy as possible premalignant lesions.

Approach: From among surgical specimens at Oakland Path. Dept. a search will be made for all benign breast lesion cases between 1964 - 1972. An additional search will be made of older non-computerized files. Four slides will be obtained for each case and reread after they are coded to safeguard against bias. Histopathologic evaluation based on Black-Chabon grading system, plus one personal variant, will be performed. Cases will be followed by searching the Tumor Registry of the State of California. A correlation will be sought between degree of duct atypia and subsequent development of breast cancer.

Progress: In an evaluation study of breast cancer risk for a variety of atypia grades classified by Black-Chabon grading system, most of the preliminaries, including assembly of slide collection from the Oakland Kaiser Hosp. and a training period for scoring and evaluation of agreement between readers, is by now completed. By the end of first project year some 500 cases will have been reviewed. Remaining 1500 cases will be reviewed in the next project year. A follow-up for cancer development will be done on each study case using the California Tumor Registry. Data will be analyzed to determine if a correlation exists between degrees of mammary duct atypia and subsequent breast cancer development.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 4/18/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $53,200

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator. Jack Fishman, Ph.D.
Name/Address Montefiore Hospital and Medical Center
Performing 111 East 210th Street
Organization: Bronx, New York

Contract Number:
Starting Date: Expiration Date:

Goal: To evaluate the possible role of genetic factors in the expression of specific biochemical parameters in breast cancer pathophysiology.

Approach: Endocrine assays will be done on blood and urine of premenopausal females from families with a high incidence of breast cancer and from matched control families. Blood will be assayed for FSH, LH, E_3, E_2, E_1, progesterone and prolactin. Urine will be assayed for 11 to 13 estrogenic, androgenic and corticosteroid metabolites. An attempt will be made to correlate familial susceptibility to breast cancer with endocrine parameters.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Bernice Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/29/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $137,[illegible]

CONTRACT RESEARCH SUMMARY

Title: Investigations of Histocompatibility Antigens and Other Genetic Polymorphisms in High and Low Risk Breast Cancer Families

Principal Investigator: Frans J. Cleton, M.D.
Name/Address The Netherlands Cancer Institute
Performing Amsterdam, The Netherlands
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high risk groups in the population for the development of breast cancer.

Approach: HL-A haplotypes will be studied in families with a high incidence of breast cancer. A large number of other genetic markers will also be investigated, such as blood groups, immunoglobulins, red cell isoenzymes and chromosomal polymorphisms. As a peripheral project, group specific antigens will be studied using a sensitive micro-immune diffusion technique with anti-mouse MTV sera and milk of lactating family members will be collected for study of a possible human MTV using electron microscopy and biochemical techniques.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date:
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $74,100

CONTRACT RESEARCH SUMMARY

Title: Role of Diet and Nutritional Status on Incidence, Pathogenesis and Natural History of Breast Cancer

Principal Investigator: Dr. Frits deWaard
Name/Address Queen Wilhelmina Fund
Performing Amsterdam, The Netherlands
Organization:

Contract Number: N01-CB-33885
Starting Date: June 28, 1973 Expiration Date: 6/27/75

Goal: To determine to what extent differences in world-wide age specific breast cancer incidence curves can be accounted for in terms of simple anthropometric measures of obesity.

Approach: Data will be collected on height and body weight of all new breast cancer cases and of control population samples in three areas of the Netherlands covered by population based cancer registries. With these data the contractor will compute age-specific breast cancer incidence rates for women of various degrees of overweight (weight-height comparisons). He will also plan similar studies in some other countries varying widely in breast cancer incidence. The hypotheses will be tested that the shape of the incidence curves for lean and fat women are different; the former resembling that seen in low incidence countries, the latter resembling that seen in high incidence countries.

Progress: Systematic collection of data on parity, age at first birth, weight and height in all breast cancer cases occurring in Friesland, the Hague and Rotterdam has been attempted. The efforts have been successful so far in Friesland and the Hague (with about 90% coverage and partly successful in Rotterdam (70% coverage). Steps have also been undertaken to ensure control groups from these populations. Visits have been made to Cancer Registries in Manitoba and Saskatchewan (Canada), Kingston (Jamaica), Sao Paulo and Recife (Brazil) to plan similar studies in these countries.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: **Bernice T. Radovich, Ph.D.**
Program: Breast Cancer Epidemiology Site Visit Date: 4/4/73
Technical Review Group: **Breast Cancer Epidemiology Committee**
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $18,500 est.

CONTRACT RESEARCH SUMMARY

Title: Biochemical and Physiological Investigations Based on Familial Genetic Patterns

Principal Investigator: Name/Address Performing Organization:	David E. Anderson, Ph.D. The University of Texas System Cancer Center Houston, Texas

Contract Number:

Starting Date: Expiration Date:

Goal: To measure aryl hydrocarbon hydroxylase (AHH) levels in members of high and low risk breast cancer families for possible use as a genetic marker.

Approach: Assay techniques will be applied on cultured lymphocytes from fresh human blood specimens, to measure for activity and inducibility of aryl hydrocarbon hydroxylase (AHH). Comparisons would be made between approximately 70 women with premenopausal (and/or bilateral) breast cancer and matched controls; also between 64 women with postmenopausal (and unilateral breast cancer) and matched controls.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: Bernice T. Radovich, Ph.D.

Program: Breast Cancer Epidemiology Site Visit Date:3/30/74

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY74 Funds: $81,515

CONTRACT RESEARCH SUMMARY

Title: Investigation of Genetic Polymorphisims in High and Low Risk Breast Cancer Families

Principal Investigator:	**David E. Anderson, Ph.D.**
Name/Address	University of Texas System Cancer Center
Performing Organization:	**Houston, Texas**

Contract Number:
Starting Date: **Expiration Date:**

Goal: To investigate a series of polymorphic genetic markers in high and low risk breast cancer families which might assist in the definition of high risk individuals.

Approach: The ABO, Rh, MNS, P, K, Fy^a and Jk^a blood group types; serum haptoglobin types; alkaline phosphatase and phosphoglucomutase red blood cell types; and chromosome abnormalities and/or variants will be determined from blood specimens. For the genetic polymorphism study a comparison will be made of 134 women with breast cancer from high risk families and normal control women matched for menopausal status, age and race. Another comparison will be between the 122 unaffected sisters of patients from high risk families and matched controls. For the chromosomal studies premenopausal and postmenopausal patients with breast cancer from high risk families will be compared with matched controls.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 5.14)

Project Officer: **Bernice T. Radovich, Ph.D.**
Program: Breast Cancer Epidemiology Site Visit Date: 3/30/74
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $41,614

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer and Estriol Dynamics

Principal Investigator: Christopher Longcope, M. D.
Name/Address Worcester Foundation for Experimental Biology
Performing
Organization: Shrewsbury, Massachusetts

Contract Number: NO1-CB-33902
Starting Date: June 1, 1973 Expiration Date: 5/31/75

Goal: To determine the production and metabolic clearance of estriol in relation to high and low excretors, in order to determine the physiological basis for repeated differences between high and low breast cancer-prone individuals

Approach: In this research the investigator plans to apply proven techniques to determine the sources and blood production rates of estriol in 1) normal women with high and low urinary E_3/E_2+E_1 ratios; the women to range in age from 21 to 90 years of age and to be nulliparous as well as parous; and 2) women with breast cancer. The aim is to determine the pattern of precursorestriol relationship and blood production rates in normal women who have high or low urinary E_3/E_2+E_1 ratios, and in women with breast cancer and to correlate the dynamics as determined by measurements made from the free estriol pool in blood with those measurements made from estrogen metabolites in urine. These data will be used to delineate better the normal dynamics of estriol production and metabolism and the role of estriol in human breast cancer.

Progress: In the first months of the contract the necessary methodology has been devised and tested for sensitivity, precision and purification for further work. From pulse injection studies, the metabolism of estriol has been characterized and the proper conditions determined for the constant infusions. Infusions have already been carried out on 6 subjects with the proper urinary ratios and these samples are currently being analyzed. Further subjects are being identified and will be infused in the ensuing months. Data will then be analyzed and the results correlated.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Epidemiology Site Visit Date: 3/23/73
Technical Review Group: Breast Cancer Epidemiology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: Role of the Stroma in the Growth of Neoplastic and Preneoplastic Lesions of the Mammary Gland

Principal Investigator: Peter O. Kohler, M.D.
Name/Address Performing Organization: Baylor College of Medicine
Houston, Texas 77025

Contract Number:
Starting Date: Expiration Date:

Goal: Determine the relationship between the stroma and the epithelial cells in the adult mammary gland, particularly in relation to epithelial growth, neoplastic or normal.

Approach: 1) Develop mammary cell culture systems to examine the role of mesenchymal cells on mammary development. 2) Examine *in vivo* in the mouse the specificity of mammary fat depots. 3) Study the relationship of different developmental stages of stroma in regulating growth of normal and preneoplastic mammary gland *in vivo*. 4) Examine the specificity of stromal cells selected from the *in vivo* mouse studies from different developmental stages and different locations on growth and differentiation of normal, preneoplastic and neoplastic mammary cells in culture.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 3)
Develop the means to prevent progression of precancerous cells to cancers through studies of cell-cell interactions.

Project Officer: D. Jane Taylor, Ph.D. and Richard A. Knazek, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/15/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $76,427 est.

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: R. David Cole, Ph.D.
Name/Address University of California
Performing Berkeley, California 94720
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones.

Approach: 1) Demonstrate that mouse mammary cell line NMuMG will respond t
hormones in some of the ways characteristic of lactogenesis in vivo.
2) Determine the effects of hormone treatment individually and in combinati
upon chromatin assembly in order to see how hormones change the program of
constructing a new pattern of genetic expression. 3) Studies of the maturation of chromatin by fractionating it according to the degree of condensati

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)
Investigate cellular and organismal modifiers of the transformation and promotion process in order to develop the means to prevent transformation of normal cells to cells capable of forming cancer.
Project Officer: D. Jane Taylor, Ph.D. and E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Hormonal Requirements of Normal and Abnormal Human Mammary Tissue in Organ Culture

Principal Investigator: Joel J. Elias, Ph.D.
Name/Address University of California
Performing San Francisco, California 94122
Organization:

Contract Number: N01-CB-23861
Starting Date: June 19, 1972 Expiration Date: 6/18/75

Goal: 1) To find conditions to maintain growth of normal, dysplastic and neoplastic human mammary tissue in organ culture. 2) To see whether morphologic classification of human mammary lesions can be improved so as to be of prognostic value.

Approach: Human mammary tissue from biopsies, mastectomies and mammoplasties will be utilized for the following studies: 1) Electron microscopic, scanning electron microscopic and light microscopic preparations of normal and abnormal human mammary tissue to define histological, structural and surface characteristics. 2) Organ culture and eventually cell culture of material studied in (1). Analysis of conditions necessary for promoting growth. 3) Effects of hormones on organ cultures studied with EM, SEM and LM. Correlation with biological behavior in vivo.

Progress: Recent success in obtaining organ cultures of mammary carcinomas that grow or remain viable for 17 days is a major step in efforts to study hormonal characteristics of individual cancers in vitro. Induction of hyperplasia and squamous metaplasia by insulin indicate that human mammary epithelium may undergo epidermal-like differentiation. From SEM photos of ductal epithelium of infiltrating ductal carcinomas clumps of microvilli of varying lengths have been found. These villi appear peculiar to carcinomas and were not seen in fibroadenomas or dysplasias. This lead will be followed to determine diagnostic value.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Efficient growth of normal and abnormal mammary tissue in vitro is an indispensable necessity for improving knowledge of cause, prevention and possibly treatment of the disease.
Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/9/72
Technical Review Group: Breast Cancer Experimental Biology Committee
[illegible] Review Group: Breast Cancer Steering Committee
[illegible] $70,000 est.

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Tumors in Dogs

Principal Investigator: Name/Address Performing Organization:	Leslie J. Faulkin, Ph.D. University of California Davis, California 95616

Contract Number: N01-CB-90033
Starting Date: August 21, 1969 Expiration Date: 10/31/74
Goal: To follow the pathological history of the mammary gland in beagles, with particular emphasis on preneoplastic lesions.

Approach: Beagles of an AEC colony kept during their life span after various treatment. 1) Glands placed in Tellyesniczky, defatted, stained in H&E, dehydrated, cleared in toluene, stored in methylsalicylate. Nodules searched with dissecting microscope, counted, and histologically classified. 2) Frequency and type of lesions correlated with age and effects of ovariectomy. Age 1-13 years. 3) Effect of ligation of mammary ducts in virgin and mated subjects. 4) Transplant of suspected dog lesions in immunosuppressed mice and hamsters or in contralateral dog fat pad cleared before estrus was established. 5) Circulating levels of progestins, luteinizing hormone and prolactin under various conditions determined. 6) Examination of milk specimens in search for viruses. 7) Culture of mammary tissue.
Progress: Following the studies of whole mounts of mammary glands which showed a large number of atypical lesions present before the development of mammary tumors, studies of factors which influence the appearance of the hyperplastic lesions were initiated. Periodic palpation of the mammary glands indicate that a number of palpable lesions exist for a long period of time and about 20 percent regress. Regressions do not correlate with the estrus cycle of the dog. Study of the neoplastic potential of lesions in vitro and in vivo are underway. Tissues have been transplanted to immunologically supressed dogs, most of these were maintained in culture but failed to grow, on the other hand, tumor tissue proliferated within an 8-day test period. The technique provides a rapid assay for determining the neoplastic potential of the hyperplastic mammary lesions. Nude-athymic mice are being used as well as ALS supressed hamsters and auto-transplantation in utilaterally mammectomized dogs. Forty samples of milk from lactating dogs have been searched for virus particles, one was found in one of the 40 samples. Attempts to find RNA dependent DNA polymerase activity in 12 of the milk samples were negative.
Significance for Cancer Research (NCP Objective 3 Approach 1)
To determine ontogeny of precancerous lesions and to delineate interaction of precancerous lesions with the host.

Project Officer: D. Jane Taylor, Ph.D., Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/8/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $75,000 Terminal

CONTRACT RESEARCH SUMMARY

Title: Human Mammary Dysplasia Study

Principal Investigator: S. R. Wellings, M.D.
Name/Address University of California
Performing Davis, California 95616
Organization:

Contract Number: N01-CB-81461
Starting Date: June 28, 1968 **Expiration Date:** 6/27/74
Goal: To characterize morphologically, functionally, and etiologically, precancerous lesions of human mammary gland.

Approach: Human mammary glands from autopsy and surgery are used for the following studies: 1) Subgross morphology of intact human breast using macrosections of dehydrated, defatted, stained, whole gland. 2) Electron microscopic (EM) analysis of mammary tissue. 3) Organ and cell culture to test response of mammary tissue to hormones. 4) Integration of morphological studies with viral, immunological and epidemiological progams. 5) Subgross analysis of 50 more mammary glands. 6) Termination of subgross atlas. 7) Larger effort in EM and culture studies of human precancerous lesion.

Progress: The manuscript entitled "An Atlas of Subgross Pathology of the Human Breast with Special Reference to Possible Precancerous Lesions" has been submitted to the JNCI. Certain atypical lobules which resemble precancerous hyperplastic alveolar nodules (HAN) of high tumor strain mice were identified in the studies which included over 150 whole human mammary glands. A colony of athymic-nude mice has been established in order to study the growth of preneoplastic lesions from human breasts. Preliminary results suggest that human mammary lobules can survive in the athymic mice, and females support the lobules better than males.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Results of these studies should aid in the diagnosis and classification of breast cancer lesions and provide data on precancerous lesions in the human breast.
Project Officer: D. Jane Taylor, Ph.D., Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology **Site Visit Date:** 2/7/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: Terminal

CONTRACT RESEARCH SUMMARY

Title: Neoplastic Potential of "Preneoplastic Lesions of the Human Mammary Gland"

Principal Investigator: S. R. Wellings, M.D., Ph.D. and
Name/Address Hanne Jensen, M.D.
Performing University of California Medical School
Organization: Davis, California 95616

Contract Number:
Starting Date: Expiration Date:
Goal: Define the growth potential of hyperplastic-dysplastic lesions of the human mammary gland also termed "preneoplastic" or "latent cancer cells".

Approach: Analysis of the growth potential of human mammary heterotransplant in athymic mice. Transplants of ducts, lobules, carcinomas _in situ_ and evident carcinomas will be implanted into female and male mice. The site of the implant will be the cleared-fat pad as well as intraperitoneally and subcutaneously. Also some transplants will be done with primary cultures following brief _in vitro_ cultivation of the lesions. Vascular and inflammatory response of the host to the transplants will be determined and the difference between biopsy and autopsy material will be assessed. The hormonal milieu of the host will be manipulated in reference to growth capacity of transplants.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4,5 Approach 2,4)
Develop methods for detecting, locating and following microfoci of precancerous and cancerous cells and improve diagnostic procedures for cancer and localization of lesions.
Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/11/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $152,828 est.

CONTRACT RESEARCH SUMMARY

Title: Role of Prolactin in Breast Cancer

Principal Investigator: Olof Pearson, M.D., and
Name/Address Jack Pensky, Ph.D.
Performing Case Western Reserve University
Organization: Cleveland, Ohio 44106

Contract Number: NO1-CB-23859
Starting Date: June 26, 1972 Expiration Date: 6/25/75
Goal: To find specific receptor sites for prolactin in breast cancer tissue which might predict hormone responsiveness of individual breast tumors.

Approach: Human and animal mammary tumor tissue for the following studies:

1) Isolation and purification of human prolactin for radioimmunoassays.
2) Development of a specific immunoassay for human prolactin.
3) Isolation of membrane fraction from mammary tissue of pregnant mice. Incubation of this fraction with ^{125}IHPr + varying concentrations of unlabeled ovine prolactin. Presence of radioactivity on the cell membrane which can be displaced by unlabeled prolactin will be taken as evidence of specific binding. 7,12-DMBA tumors will also be used.
4) Measurement of prolactin levels in serum and other biological fluids in breast cancer patients.

Progress: Prolactin (HPr) has been isolated and purified up to 70% from 150 human pituitaries, its growth hormone content is less than 1%. A radioimmunoassay for prolactin had been developed with a sensitivity to 1 to 2 ng/ml. HPr levels have been determined in breast cancer patients before and after therapy. HPr in cancer patients did not vary significantly from the normal controls and the level rises in both groups during sleep. A new agent (Lilly) suppresses HPr for 24 hours in humans and has good effects in suppressing growth of DMBA-induced rat mammary tumors. Prolactin receptors have been found in the rat mammary tumors and are now being measured in human breast tumors.

Significance for Cancer Research (NCP Objective 5 Approach 6)
An approach directed toward the understanding of the role of the hypophysis in the growth of breast cancer. It is important to determine the presence of prolactin receptors and what their relations may be to estrogen receptors.
Project Officer: D. Jane Taylor, Ph.D. and Harold Heitman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/18/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $73,000 est.

CONTRACT RESEARCH SUMMARY

Title: Biochemical Nature of Hormone Dependency in Breast Cancer

Principal Investigator: Name/Address Performing Organization:
Eugene DeSombre, Ph.D.
University of Chicago
Chicago, Illinois 60637

Contract Number: N01-CB-23873
Starting Date: June 1, 1972 **Expiration Date:** 5/31/75

Goal: 1) To assess the effect of estrogen binding on the RNA polymerase activity of nuclei from hormone dependent and independent tumors. 2) To evaluate th biological meaning of the cytosol estrophile transformation from the native 4S unit to the 5S form.

Approach: Mammary tumors of Sprague-Dawley female rats induced with 7,12-Dimethylbenzanthracene for the following studies: 1) Separation of 4S and 5S cytosol estrophiles and determination of their binding capacity. Solubilization of nuclear receptors and their characterization by sedimentation analysis. Comparison between 8S and "salt stable 4S" estrophiles. 2) Use of temperature as a screening parameter for detecting binding capacity of nuclear fraction. 3) Analysis of effects on RNA-polymerase activity of the 5S estrophile complex isolated from hormone dependent and independent tumors exposed to estradiol. 4) Attempts to separate in the cytosol the factors which transform 4S into 5S component of the estrophile proteins. 5) Attempts to block tumor growth by depriving the nuclei of the 5S estrophile complex with estrogen which appears essential for RNA synthesis.

Progress: A stable 4.5S and nuclear 5S uterine receptor was differentiated by gel electrophoresis. The role of the stable 4.5S form has not been established it is sometimes found in the cytosol of human breast cancer specimens. Nuclei of hormone independent tumors take up estrogen receptor (ER) complex from cytosol of hormone dependent tumors. ER complex stimulates RNA polymerase activity both in vivo and in vitro. There is no RNA polymerase activity when tumor cytosol lacks ER. Nuclei of hormone independent tumors were not stimulated. Parke-Davis CI-628 anti-estrogen can prevent the affinity of estrogen with cytosol receptors. CI628 binds to 8S complex of uterine cytosol, however, there is a greater affinity to bind to non-specific 4S component. Chronic treatment of DMBA-induced tumors with CI628 effects regression of 60 percent of the tumors which is comparable to ovariectomy response. A late response of uterus to estrogen is induction of peroxidase activity. This was also demonstrated in hormone dependent tumors. This could be useful to differentiate hormone dependent from independent breast cancers.

Significance for Cancer Research (NCP Objective 3 Approach 3)
The project looks at one aspect of the role of hormones in tumor growth. Is important to pinpoint basic biological difference between hormone responsive and hormone non-responsive tumors. Could provide leads to treatment of disease.

Project Officer: D. Jane Taylor, Ph.D., E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/21/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $100,000 est.

CONTRACT RESEARCH SUMMARY

Title: Identification of Cell Types in Cultures of Normal and Neoplastic Breast Tissues

Principal Investigator: Samuel Abraham, Ph.D.
Name/Address Children's Hospital
Performing Oakland, California 94609
Organization:

Contract Number: NO1-CB-33906
Starting Date: June 27, 1973 Expiration Date: 6/26/75

Goal: To identify cell markers in normal and neoplastic breast cultures of mouse, dog and human origin.

Approach: Antisera to be prepared using whole cells, membranes and microsomal preparations as antigens. Adipose and fibroblast cells to be obtained from cleared fat pad, thus separation for the epithelial cells. Methods of Abraham to be used for separating parenchymal from stromal cells of the mammary gland. Anti-myosin serum to be used for identifying myoepithelial cells. Antibodies to be conjugated with peroxidase and reacted with different color producing substrate. Attempts to characterize the nature of cultured cells by the color assumed by each cell type to be made. Fibroblasts to be identified by activities of proline hydroxylase and lysyl oxidase. Lactose synthetase B protein to be purified and an antiserum produced for detecting the presence of protein in epithelial cells. The multienzyme complex fatty acid synthetase, may be unique to epithelial cells, if so, an antibody will be prepared and used as a discriminant. Possibility of using unique isoenzyme to distinguish normal from neoplastic cells to be explored. Immunological specificity for cell identification is the method of choice.

Progress: Two techniques have been developed for isolation of cells, one is more successful for normal cells, the other for tumor cells. The integrity of the isolated cells has been determined by in vitro cultivation and biochemical techniques. Immunological studies with freshly isolated and cultured cells have been initiated: work with cell surface antigens have led to an investigation of their nature and biosynthesis; casein and α-lactalbumin were chosen for study of secretory product antigens; and prolactin is under study in special receptor tests. Biochemical studies of the cell surface antigens have been initiated.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Finding a marker for each cell type within a heterogeneous tumor would be helpful to the pathologist as well as the cell biologist and biochemist who must study the products of the whole tumor as produced by the individual cell types.

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Cancer Experimental Biology Site Visit Date: 3/12/73
Technical Review Group: Breast Cancer Experimental Biology Committee
[illegible] Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies to Determine the Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigator: Susumu Ohno, D.V.M., Ph.D., D.Sc. and
Name/Address James Kan, Ph.D.
Performing City of Hope Medical Center
Organization: Duarte, California 91010

Contract Number: N01-CB-33907
Starting Date: June 27, 1973 Expiration Date: 6/26/75
Goal: To study the consequence of the testicular feminization mutation on the mammary gland and the effect of androgen "insensitivity" on the development of the secretory patterns of pituitary gonadotrophins.
Approach: A colony of Tfm/Y ⚥ to be examined for number and conformation of nipples and breast development. Tfm/O ♀ will be mated, colostrogenesis, lactogenesis, and litter growth, to be followed. Behavior of Tfm mammary gland cells in a male environment. Sex reversion of Tfm/+ heterozygotes to males to be obtained by introduction of Sxr mutant gene which gives testes and male phenotypes to X/X and X/O females. Mediation of Tfm regulatory locus on imprinting female or male secretory pattern on the hypothalamus-pituitary axis to be studied by analyzing; a) LH and prolactin production by estrogen treatment of Tfm/Y ⚥ ; b) influence of testosterone given to new born on LH and prolactin production; c) level of androgen binding by polysomes in the cytosol or by nuclear fractions, using testosterone, DHT and 5α-androstan-3α-17β-diol; d) estrogen receptor neurons and androgen binding protein in the hypothalamus at neonatal stage. Role of testosterone in mammary cell death to be studied also in a tumor which is testosterone dependent for growth *in vivo* but not *in vitro*. Mutants such as Tfm/Y ⚥, Tfm/O ♀, Tfm/+, Sxr/+ ♂, and Tfm/O, Sxr/+ ♂, derived from a Tfm for mammary tumor presence.
Progress: Studies on Tfm mice have proceeded, including identification of an X-linked site which exerts a position effect on the Tfm mutant. Efforts on the pituitary-hypothalamic axis, on preferential X-inactivation and steroid receptors in Tfm animals have developed commendably. The senior investigators have published three papers, another is in press and two others have been submitted to journals. All of the papers concern Tfm and the new mutation O^{hv}.

Significance for Cancer Research (NCP Objective 1 Approach 4)
Study of an unusual animal model which may contribute valuable information to the etiology of hormone responsive mammary cancer.

Project Officer: D. Jane Taylor, Ph.D., E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/10/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $95,000 est.

CONTRACT RESEARCH SUMMARY

Title: Protein Nucleic Acid Interactions in Transcription Controls of Normal and Malignant Cells

Principal Investigator: Kenneth S. McCarty, Ph.D.
Name/Address
Performing
Organization: Duke University
Durham, North Carolina 27710

Contract Number: N01-CB-23857
Starting Date: June 29, 1972 Expiration Date: 6/28/75
Goal: To isolate, purify and fractionate nuclear acidic, nonhistone, proteins from mammary gland nuclei.

Approach: C_3H mammary gland and C_3H mammary adenocarcinoma, normal and leukemic lymphocytes and duck erythrocytes will be employed in the following studies. 1) Isolation of chromatin. 2) Extraction of acidic proteins. Published techniques to be perfected. 3) Binding capacity of extracted acidic proteins to DNA. Reconstitution of chromatin. 4) Capacity of reconstituted chromatin to transcribe specific messages. 5) Consequences of acetylation, phosphorylation and methylation on template activity of reconstituted chromatin.

Progress: A technique for rapid large scale preparation of nuclei using a density gradient rotor with continuous sample flow has been developed and published. A procedure for purification and fractionation of acidic and neutral chromosomal proteins is almost ready for publication. Studies of hormonal action on acidic chromosomal protein 90 to 95 percent of ^{32}P was found to bind and ^{32}P was incorporated only in the F_{2a2} faction and only in the S phase. A partial fractionation of phosphorylated thymus chromosomal proteins related to hormone stimulation has been observed in cultures of mammary gland and mammary cultures.

Significance for Cancer Research (NCP Objective 3 Approach 3)
To increase knowledge of acidic chromosomal protein properties is indispensable to advance our comprehension of hormone responsiveness of breast cancer.

Project Officer: D. Jane Taylor, Ph.D., E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date:
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $52,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3',5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator: David F. Scott, Ph.D.
Name/Address Performing Organization: Medical College of Georgia
Augusta, Georgia 30902

Contract Number: N01-CB-33909
Starting Date: June 30, 1973 Expiration Date: 6/29/75
Goal: Activity levels of 3',5'-cyclic adenosine monophosphate and the enzymes responsible for its formation and degradation will be correlated to growth rate of mammary tumors to see whether any meaningful generalization can be made.

Approach: Adipose, ductal and alveolar cells of normal and developing mammary gland and neoplastic cells of mammary tumors will be isolated. The activity of adenylate cyclase and phosphodiesterase will be measured in homogenates of whole glands, in isolated cells, in mammary gland explants and in cultures of each cell type. The hormonal regulation of cAMP levels will be studied using the adenosine prelabeling procedures as developed by Kuo and by Schultz and Daly. C_3H and A mice will be used first, human tissue later. Mice with normal hyperplastic nodules and carcinomas will be obtained under the supervision of Dr. A. G. Liebelt. Cell isolation and cultures will be carried on under the supervision of Dr. E. Howard. Growth rate and cAMP levels will be compared in tumors and in normal cells. The main objective is to understand the mechanism(s) by which cAMP levels are controlled in mammary tissues and isolated cell preparations, both adipose and epithelial.

Progress: Preliminary studies indicate that mouse mammary epithelial cell in explant cultures respond to prolactin, epinephrine or prostaglandin E_1 with elevated cAMP production. Attempts are being made to obtain established cell lines of tumors and normal tissue and success has been made in subculturing normal cells for several passages. One tumor cell line has been established.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Study of enzyme systems which may contribute important information on the cause of the disease and to possible methods of treatment and prevention.

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $80,000 est.

CONTRACT RESEARCH SUMMARY

Title: Cellular Control in Mammary Involution and Dedifferentiation

Principal Investigator: **Bruce L. Larson, Ph.D.**
Name Address University of Illinois
Performing Urbana, Illinois 61801
Organization:

Contract Number: N01-CB-23856
Starting Date: June 28, 1973 Expiration Date: 6/27/75
Goal: To enhance our knowledge of dedifferentiation of neoplastic cells by studying the process of involution of the normal mammary gland _in vivo_ and loss of differentiation of lactating mammary cells _in vitro_.
Approach: Using lactating mammary glands of cows and rats and the mammary carcinoma R3230AC which still has the capacity to reproduce small amounts of milk constituents. 1) Secretory mammary cells placed _in vitro_ culture and production of milk constituents and other unique lactation functions studied while cells are changing from lactating to proliferating units. 2) Biological and biochemical characteristics of degenerating cells studied during mammary gland involution both normal with progression of lactation and that induced by the cessation of milk withdrawal. 3) Mammary glands of rats and cows studied to correlate differences in mechanism(s) of cellular growth of the secretory cells and subsequent regression during involution. (The major proliferation of secretory cells in cows occurs during pregnancy which have a long lactation. Cows have few mammary tumors as compared to rats. (Is the involution process different?)

Progress: In dispersed cultures of bovine mammary gland the rate of lactose synthesis was found inversely related to cell density. Lactose added to the medium did not affect lactose synthesis but B-lactoglobulin and orotic acid did. Accumulation of milk _in vivo_ may result not only by feedback accumulation of milk but also by exposure of the whole cell surface to milk constituents as a consequence of milk accumulation. Glucose-6-phosphate dehydrogenase used as a lactation marker is not reliable since the bands are not isoenzymes but interconvertible molecular forms. Several forms of rat α-lactalbumin have been isolated.

Significance for Cancer Research (NCO Objective 3 Approach 1)
Basically this is a study of the nature and modification of mammary tissue, normal and abnormal, which should form a basis for developing means to prevent conversion of cells to those capable of forming cancers.
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/17/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $65,000 est. Terminal

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Name/Address Performing Organization:	Klaus Kratochwil, Ph.D. Institute for Molecular Biology Salzburg, Austria

Contract Number: N01-CB-33883
Starting Date: June 29, 1973 Expiration Date: 6/28/75
Goal: To study the acquisition of hormone responsiveness during development of the mammary gland.

Approach: Previous work has demonstrated that the mammary gland develops along the female-type unless androgens are produced by the fetus. Action of testosterone on the mammary gland will be analyzed to establish whether sensitivity to the hormone exists during the whole developmental period or whether it is acquired at any given time. This generation to be studied by explanting mammary gland buds in several areas of the same embryo or in other embryos at various stages of development. Determination of the developmental stage to be followed by evaluating binding and concentration of testosterone to the mammary tissue. Role of the stroma in determining the androgen-induced atrophy of the mammary gland to be studied by separation and recombination of epithelial and mesenchymal components from different subjects at different stages of development. EM analysis of the epitheliomesenchymal interface to be done. A morphological comparison between the appearance of regressing mammary cells and the effects of collagenase, hyaluronidase, actinomycin D, and cycloheximide on the destruction of mammary rudiments to be carried on as a preliminary study to understand the mechanism of cell death due to testosterone action.

Progress: The contract was not initiated until August 1973 because of delay in arrival of notice of the award and immediately experienced difficulty with devaluation of the dollar. However the project is now ongoing and results are being obtained. Experiments to date confirm earlier results that the embryonic rudiment mouse mammary gland is not yet responsive to testosterone at the time of formation from the epidermis. Rudiment acquires its responsiveness only at about 13 1/2 days of gestation and loses it on day 15 of fetal life. Hormone specificity of the response has been verified also with other androgenic and non-androgenic steroids. Initial studies have been done on tissue interaction involved in the androgen response. EM studies indicate a dramatic change at the epithelio-mesenchymal interface at the beginning and during androgen destruction of epithelium.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Should provide very basic information on the development of hormonal responsiveness in normal and neoplastic cells.

Project Officer: D. Jane Taylor, Ph.D., E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 9/20/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $32,000 est.

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acid Preparations on the Biological Properties of Mammary Carcinomas

Principal Investigator: Aaron Bendich, Ph.D.
Name Address
Performing
Organization: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

Contract Number
Starting Date: Expiration Date:

Goal: To determine the relationship between transfer of nucleic acid preparations into mammary cells and possible changes in their growth potential.

Approach: 1) Functional transfer of genetic activity to somatic cells using sperm as the vehicle. Analysis of "turning on" of embryonal gene expression such as fetal antigens. 2) Functional transfer of genetic activity to somatic cells using isolated chromosomes at metaphase. 3) Functional transfer of genetic activity to somatic cells using isolated DNA. 4) Exposure of enucleated cells to DNA and DNA-containing moieties (sperm). Cytochalasin B will be used as the enucleating agent.

Cell lines of human and animal mammary, normal and neoplastic, will be employed along with lines of other origin.

Progress: New Contract.

Significance for Cancer Research (NCP Objective 2,3 Approach 3,3)
Alter genetic makeup or gene expression to reduce the rate of cancer development. Investigate cellular and organismal modifiers of the transformation and promotion process.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/22/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $79,738 est.

CONTRACT RESEARCH SUMMARY

Title: Biological Characterization of Animal Mammary Tumors and Tumor Bank Facility

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address Mason Research Institute
Performing Worcester, Massachusetts 01608
Organization:

Contract Number: NO1-CB-31146
Starting Date: June 28, 1963 Expiration Date: 6/27/75
Goal: To maintain a bank of biologically characterized and monitored, transplantable mammary tumors to be used by the BCTF and other selected investigators.

Approach: Freeze-preservation of mammary and endocrine related tumors: 1) submittal of tumors by qualified investigators able to furnish pertinent background information; 2) tissues tested for a)viability and b)biologically characterized, both pre-and post-freeze-preservation, by determination of growth curves, specific effects on host organs, host survival time, serum hormone levels, histology, karyology, immunogenicity and response to ovariectomy; 3) characterized tissues preserved in Linde liquid nitrogen freezers according to well recognized, proven procedures.

Progress: Sixty tumors have been shipped to 39 different investigators in the U.S. and 5 foreign countries reflecting an increase in shipments since 1966. Thirty-seven percent of the requests were for tumors of mammary origin and twenty percent were anterior pituitary tumors characterized by ACTH, prolactin, and/or growth hormone secretion. The secretory activity of the anterior pituitary tumors MtT/F4, MtT/W5, MtT/Wf/StH, MtT/W15 and MtT/F45 are being verified by determining serum hormone levels and effects on target organs in rats bearing these tumors. The 13762 mammary adenocarcinoma has been converted to the ascites form with two sublines established in serial transplantation providing yields of 20 ml of ascites per rat with ascitocrits > 25% packed cells. The objective is to furnish investigators in cellular membranes and metastases with natural cell suspensions of mammary tumor origin.

Significance for Cancer Research (NCP Objective 3,4,5 Approach ______)
Facilitates the reproducibility of basic research by serving as a repository and reference source for experimental materials and by providing investigators with biologically characterized and monitored neoplastic tissues for animal studies.
Project Officer: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/4/71
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Dwight R. Robinson, M.D.
Name/Address Performing Organization: Massachusetts General Hospital
Boston, Massachusetts 02114

Contract Number: NO1-CB-33918
Starting Date: June 30, 1973 Expiration Date: 6/29/75
Goal: To determine whether there is a difference in prostaglandin (PG) production in human mammary tissue, normal and neoplastic.

Approach: Production of PGE, PGF_1 (1 and 2 alpha) to be measured in culture medium of human neoplastic breast tissue incubated for various time after surgical removal. Attempts to obtain organ cultures of normal tissue from the same gland. Effect of antitumor agents to be tested on PG production by breast tumors. Levels of cAMP to be measured in the culture fluid and radioimmunoassays to be used for PG and cAMP determinations. Level of PG to be measured in serum of patients and attempts to measure PG in the efferent blood of the tumors. PG production and cAMP levels to be measured in culture medium of 2 clonal tissues - rat mammary tumor and ovary, a correlation will be sought between PG production and initiation or arrest of growth. These lines and synovial fibroblasts will be a reference point for studies of human material based on previous results. Blocking and resumption of PG production with Indomethacin to be studied in human samples and correlated with growth of cultured tumor cells.

Progress: Procedures have been developed for obtaining surgical tissue specimens. Fourteen breast cancers, 6 benign lesions and 3 normal breast specimens have been sucessfully cultured. Medium in which these specimens have been maintained have been frozen for analyses. Radioimmunoassay procedures (Levine) for PG have been set-up and 150 assays per week can be done. Preliminary results for PGF and PCB have been obtained on human tissue but it is too soon to draw meaningful conclusions.

Significance for Cancer Research (NCP Objective 4 Approach 1)
A study of a parameter of the biology of the cell (mammary) which should lead to new information never before studied.

Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/14/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 74 Funds: $62,201 est.

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and Influence Cellular Biochemistry

Principal Investigator: Arnold Rubin, M.D.
Name/Address Mount Sinai School of Medicine
Performing New York, New York 10029
Organization:

Contract Nubmer: N01-CB-33912
Starting Date: June 28, 1973 Expiration Date: 6/27/75
Goal: To study the interaction between lymphocytes and normal or neoplastic mammary cells.

Approach: Lymphocytes from heparinized peripheal blood tested against fresh human mammary tissue, cultured cells and their sonicated extracts. Lymphocyte mediated immunity assessed by lymphocyte transformation, leukocyte migration inhibition, etc. Direct cytotoxic effects of activated lymphs assayed. Sensitivity of mammary cells to cytotoxic factor tested by modification of the Granger technique. Cytotoxic factor produced by a human lymphoid cell line and column purified, effect of factor on DNA, RNA and protein synthesis tested in autologous cell systems. Various cell types in culture evaluated by EM in relation to organelle development and to the effect produced by lymphs of effector molecules. Attempts to be made to characterize biochemically the binding site of effector molecules and to elucidate the nature of the molecules-receptor site reaction. Parameters to be measured in normal and neoplastic tissue to include: 3H thymidine uptake, RNA polymerase activity, molecular species of RNA reproduced, etc.

Progress: Several cultures of malignant, benign and normal tissue derived from 20 biopsy specimens and 3 breast milk samples have been established. Inhibitors of RNA, DNA and protein synthesis were derived from established long-term lymphoid and breast lines. The most significant inhibition of lymphocytes was seen to date with fractions from the slowest growing breast cancer line. Cell fractionation techniques have been worked out. Pure nuclei at yields of 50 to 70 percent have been prepared, from these relatively pure nuclei have been prepared.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Study cell surface and cell membranes for obtaining new information as to means of preventing the transformation of normal cells to neoplastic ones.

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/30/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which They Influence Cellular Biochemistry

Principal Investigator: Dr. Kurt E. Ebner
Name/Address Performing Organization: Oklahoma State University
Stillwater, Oklahoma 74074

Contract Number: N01-CB-33910
Starting Date: June 27, 1973 **Expiration Date:** 6/26/75

Goal: To study composition and biochemical properties of normal and neoplastic mammary cell plasma membranes.

Approach: Single cell suspensions obtained from normal and lactating mammary glands and tumors of rats using several methods. Separation of cell types using differential centrifugation and specific activity column. Synthesize such a column by binding prolactin chemically to a partially hydrolyzed nylon support. Assessment of change to the cell membranes by comparison of glucosamine ^{14}C, fucose ^{14}C and leucine ^{3}H uptake into membrane protein of freshly isolated and incubated cells which have "repaired" their damaged membranes. Similarities and differences of surface structures between mammary tumor and cell membranes evaluated by binding of ^{125}I Con. A and lectins. Isolation of cell membranes by sucrose density centrifugation of cells broken by homogenization or N_2 cavitation with or without treatment with membrane-stabilizing agents. Purification of membranes by affinity chromatography, isoelective focusing, etc. and purity assessed by various biochemical and microsopic methods. Membrane glycoproteins to be isolated for studies of composition and structure. Quantitative binding of prolactin to cell membranes to be determined.

Progress: Plasma membrane fraction from normal lactating rat mammary glands and the R3230 tumor have been prepared. A 15-20 fold purification of the membrane marker enzymes 5'-nucleotidase and Na^+, K^+-ATPase was affected. Protein and glycoprotein components of the fractions have been examined by SDS-acrylamide gel electrophoresis. Basic band patterns for the proteins appear to be the same for normal and tumor membranes. One glycoprotein band is different between normal and tumor.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Study of cell surfaces and cell membranes in order to obtain new information which would be useful in developing means to prevent transformation of normal cells to neoplastic ones.

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology **Site Visit Date:** 3/8/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Reference Review Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Characterization of Mammary Tumors

Principal Investigator: Joseph Leighton, M.D.
Name/Address Medical College of Pennsylvania
Performing Philadelphia, Pennsylvania 19129
Organization:

Contract Number: N01-CB-23858
Starting Date: June 28, 1972 **Expiration Date:** 6/27/75
Goal: To develop a protocol for predicting the growth potential of human breast carcinoma, and their reaction to different modes of treatment.

Approach: 1) Use embryonated eggs as host for surgical specimens of mammary carcinoma. Vary conditions of transplantation on the chorioallantoic membrane: combining fragments of tumor and embryonic organs; administering hormones; using abnormal temperatures of incubation. Use frozen-thawed samples of the same tumors for therapy sensitivity tests. 2) Establish monolayer cultures of human breast tumor cells by "spilling" or tissue dissociation techniques. Use monolayers in meniscus-gradient culture where distinctive patterns occur, characterized by organoid piling up adjacent to the gas phase, and a monolayer of changing cytologic character as the gradient descends. Correlate divergent morphologic patterns of similar appearing biopsies with clinical course. Examine effect of pharmacologic materials on patterns of growth. 3) Compare fibroblasts _in vitro_ from normal and neoplastic areas of mastectomy specimens as to morphologic patterns of growth and collagen formation. Determine whether varieties of stroma in different breast tumors derive from permanently altered fibroblasts or from reversible local influence of carcinoma cells on normal fibroblasts.
Progress: Human and rat mammary carcinomas have been utilized in three feasibility studies; growth of tissue transplants on chorionic allantoic membrane (CAM) meniscus-gradient cultures; on cellulose and plasma clots. These techniques have not been successful therefore studies were initiated with human breast cancer cell lines, BT-20, HBT-3 and HBT-39. Fundamental information on the structure of tumor tissue is now being obtained using these cell lines. Work has been initiated to analyze the aggregation capacity of the human cell lines.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Through development of a method for maintaining human breast cancer in the laboratory it should be possible to achieve an accurate assessment of the probable course for each patient and the best course of treatment.
Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/28/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $60,000 est. Terminal

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Human Mammary Tissue: Hormone Dependent Susceptibility of Mammary Tissue to Transformation

Principal Investigator: Edwin V. Gaffney, Ph.D.
Name/Address Performing Organization: Pennsylvania State University
University Park, Pennsylvania 16802

Contract Number: N01-CB-33898
Starting Date: June 28, 1973 Expiration Date: 6/27/75
Goal: To develop human mammary cell lines, to transform them with SV40 or benzpyrene, to study the hormonal response in vitro of human neoplastic cells.

Approach: Prepare single cell suspensions of human breast cancer using hyaluronidase,collagenase or harvesting cells present in exudates formed on the cut surface of tissue. Separate cells by sedimentation at unit gravity in bovine albumen gradients. Cultivates individual fractions by adding 1% methyl-cellulose to culture media and 7% acrylamide gel prepared as for electrophoresis and detoxified, procedure for reducing fibrobalst overgrowth. Human cell lines will be monitored for casein and lactose synthetase and attempts to transform them with SV40 and benzpyrene will be made. Response of transformed cells to hormones will be studied in terms of culture growth and DNA synthesis.

Progress: Initial experiments were aimed at designing a method of obtaining large quantities of viable cells for in vitro studies. A successful procedure has been developed using autopsy breast tissue, cell viability was > 90% from samples up to 24 hours post-mortem. Monolayer cultures were established and fibroblasts have been eliminated from cultures by maintaining cells on polyacrylamide gels for approximately a week. Human breast epithelial cultures have been obtained from milk samples. Thirty-five samples of intraductal carcinomas have been obtained. 60 percent yielded a cell population which would attach to the surfaces of the culture flasks, cell proliferation in many of these cultures declined after a week. Attempts have been made to enhance survival of the cells in vitro with hormonal supplements to the media.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Through in vitro cultivation of human mammary tissue, normal and abnormal, determine characteristics of cancer that produce different growth rates which may be influenced by viruses, carcinogens and/or hormones.

Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/28/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Referral Review Group: Breast Cancer Steering Committee
FY74 Funds. $58,000 est.

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis; A New Approach to the Study of Control of Prolactin Secretion in Relation to Mammary Cancer

Principal Investigator: Wesley C. Hymer, Ph.D.
Name/Address Performing Organization: Pennsylvania State University
University Park, Pennsylvania 16802

Contract Number: NO1-CB-23863
Starting Date: June 28, 1972 Expiration Date: 6/27/75
Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach: The following materials will be used: 1) Human adenohypophysis from autopsy and surgical material. 2) Rat adenohypophysis from normal, lactating, estrogen treated and pituitary mammotropic tumor bearing females. The experiments will include: 1) Cell dissociation with trypsin spinner suspension flasks + Pasteur pipettes expulsion. 2) Sedimentation at 1G BSA gradient chamber NIH model. 3) Equilibrium density centrifugation.

Progress: Rat pituitary cells subjected to velocity separation at unit gravity produces mammotrophs to a purity of 70% which retain their full compliment of hormone. Mammotrophs from estrogen primed rats sedimented further than those prepared from ovariectomized or diestrous rats. An analogous situation was observed with human separated mammotrophs whereby those from a control tissue co-sedimented with the main cell peak whereas specimens from breast cancer patients separated faster than the main cell peak. Difference in sedimentation could be due to hypertrophy of the mammotrope. A mammotrope cell type was identified by EM responsible for prolactin productions. Cultures of separated rat mammotrophs show that not all secrete the same amount of prolactin. The lower gradient fractions secrete 3-6 times more prolactin than those in the upper gradient. Prolactin secretion is suppressed by 50% by dopamine and ergocornine. Human pituitary cells from a diabetic patient maintained in cell culture for 30 days and the rate of prolactin secretion decreased with time. Prolactin secretion from cells from 2 breast cancer patients were initially high but fell to low levels after 12 days in culture.

Significance for Cancer Research (NCP Objective 3 Approach 3)
To further understand the role of prolactin in mammary tumor growth and to be able to interfere with tumor growth through manipulation of prolactin production one must use pure cultures of mammotrophs to analyze how cells function.

Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/3/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $75,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies of Isoenzymes in Normal, Pre-Neoplastic and Neoplastic Breast Tissues as an Approach to Define Unique Properties of Neoplasia

Principal Investigator: Russell Hilf, Ph.D.
Name/Address University of Rochester
Performing Rochester, New York 14642
Organization:

Contract Number: N01-CB-33880
Starting Date: May 21, 1973 **Expiration Date:** 5/20/75

Goal: To utilize glucose-6P-dehydrogenase and lactate dehydrogenase isoenzyme patterns to characterize normal, preneoplastic and neoplastic mouse mammary tissue.

Approach: The isoenzyme patterns will be examined in normal breast at various stages of development, during pregnancy, lactation, after parturition, in preneoplastic and neoplastic conditions. Preliminary experiments showed a sharp increase in the quantities of G6PD1 in tumors versus virginal and lactating gland. The isoenzymes for mammary tumors will be purified and antisera will be produced. A radioimmunoassay will be developed for use as a discriminant assay on the assumption that differences in isoenzyme quantities characterize neoplastic and preneoplastic tissues. These studies will be carried on in parallel with G6PD and LDH isoenzyme profiles in normal and neoplastic human tissues now supported by grants. The rodent work is done to permit an analysis of the effects of exogenous hormones and the ablation of endocrine organs on the isoenzyme profile.

Progress: Mammary gland, preneoplastic tissue (hyperplastic alveolar nodules or HANs) and tumors from BALB/c virus-free mice as well as BALB/cfNIV mice with virus are being analyzed for enzymes and isoenzymes. Preliminary results obtained on virus-free BALB/c hosts showed the activity of glucose-6-phosphate dehydrogenase (G6PD) in lactating mammary tissue elevated compared to G6PD glands of pregnant mice, the opposite was seen for lactate dehydrogenase (LDH). The data from HANs (from virgins) resemble those from pregnant gland but the enzyme levels are lower for HANs from lactating tissue. Isoenzyme of G6PD have been examined in the same tissues, two bands were obtained for both pregnant and lactating glands. In these early results it appears that HANs are capable of responding to the hormonal stimuli of pregnancy and lactation

Significance for Cancer Research (NCP Objective 4 Approach 1)
Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Project Officer **D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.**
Program: Breast Cancer Experimental Biology Site Visit Date: Deferred
Technical Review Group: **Breast Cancer Experimental Biology Committee**
Relevance Review Group: **Breast Cancer Steering Committee**
FY 74 Funds: $35,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies on the Prevention of Metastasis in Mammary Cancer

Principal Investigator: Untae Kim, M.D.
Name/Address Roswell Park Memorial Institute
Performing Buffalo, New York 14203
Organization:

Contract Number: N01-CB-23864
Starting Date: June 15, 1972 Expiration Date: 6/14/75
Goal: Determine the mechanism of breast cancer cell dissemination in relation to their plasma membrane properties and host immune states, and to develop clinically applicable procedures and rationale for prevention of metastasis.
Approach: Utilizing spontaneously metastasizing and non-metastasizing mammary carcinomas in W/Fu rats, the following studies will be done: 1) Analysis of tumor cell surface properties by: electron microscopic histochemistry; chemical composition of plasma membranes, gylcosyl transferase and glycosidase activities; plasma membrane associated 5'-AMPase, alkaline and acid phosphatases, phosphodiesterase, NAD pyrophosphatase, ATPases, and adenylcyclase; lysosomal and non-lysosomal enzymes. 2) Effects of plasma membrane modifiers, e.g., plant lectins, neuranimidase proteolytic enzymes, etc., on tumor cells using approach (1). 3) Immunoassay of circulating, plasma membrane associated tumor cell antigens. 4) Effects of membrane modifiers on the tumor host immune lymphocytes. 5) Collection of neoplastic cells from thoracic duct, vein and arteries with the aim of understanding routes of metastases and ways of interfering with them using approaches (2) and (4).
Progress: Seven metastasizing and 4 non-metastasizing tumors were established. A sub-line of DMBA-4 was established which has the unusual "self-curing" feature i.e., the metastases regress when the primary regresses. The metastases do not regress when the primary tumor is surgically removed. Ascites cell lines have been developed from most of the metastasizing tumors, only the line from TMT-081 is maintained due to its stability and cell yield. Ascitic cells acquire a ruthenium-positive glycocalyx coat which is absent in the solid tumor in vivo. SMT-2A and TMT-081 metastasizing tumors and 2 non-metastasizing tumors are being cultured in suspension. Non-metastasizing tumors are more cohesive and form clusters around latex beads while metastasizing tumors grow singly and freely in suspension and form fewer clusters. There is no significant difference in chemical composition of plasma membranes between metastasizing and non-metastasizing tumors, however, there is several hundred times activity of alkaline phosphatase, 5'-nucleotidase, phosphodiesterase, nucleotide pyrophosphatase, alkaline ribnuclease in the non-metastasizing tumors. Metastasizing tumors have higher cAMP levels and lysosomal enzymes.
Significance for Cancer Research (NCP Objective 4 Approach 3)
Immunological status of the host and metastasizing capacity of the tumor cells are two interdependent parameters. Knowledge of the biological basis of this interdependence is essential for treatment of breast cancer.
Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Studies on Basic Biological Events in the Pathogenesis of Mammary Cancer

Principal Investigator: Thomas L. Dao, M.D.
Name/Address Roswell Park Memorial Institute
Performing Buffalo, New York 14203
Organization:

Contract Number: NO1-CB-23865
Starting Date: June 15, 1972 Expiration Date: 6/14/75
Goal: Determine the role of hormones and other host factors in the pathogenesis of mammary dysplasia and neoplasia.

Approach: 1) *In vitro* experiments on carcinogenesis with organ cultures of rat mammary glands and analysis of hormonal effects on carcinogenesis. 2) Characterization of hyperplastic alveolar nodules (HAN) in the rat through transplantation, frequency in relation to neoplasia, *in vitro* behavior, hormone dependency. 3) Characterization of carcinogen induced (7,12-DMBA) tumors and HAN by estrogen receptor protein and histone acetylation of growing, regressing and regrowing specimens and HAN. 4) Definition of the relationship between prolactin and estrogen on mammary tumor growth through determination of hormone levels in blood, pituitary grafts and lesions of the medial eminence.

Progress: HAN were found more numerous in the inguinal mammary area but carcinomas are equally distributed in all glands. There is a progressive increase in number of HANs along with time after DMBA treatment, however, there is no direct relation between the number of HAN and number of tumors. HAN can be detected by transillumination and cultivated *in vitro* for a maximum of 33 days in media supplemented with hormones. Labelling indices measured in cultured normal, HAN, and tumor tissues in presence of hormones were lower in HAN however the S phase was the same for HAN and normal mammary gland. Since HAN does not have estrogen receptor protein suggests that HAN is not estrogen dependent. Ninety day old Sprague-Dawley ♀ rats are refractory to DMBA carcinogenesis, however exposure to DMBA induces as many HAN as in younger animals thus a lack of relationship between HAN formation and tumor induction.
Significance for Cancer Research (NCP Objective 3 Approach 1)
Through studies of the nature of oncogenic cell transformations in carcinogenesis of mammary tissue develop means to prevent conversion of cells to those capable of forming cancer.
Project Officer: D. Jane Taylor, Ph.D., Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/20/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $70,000 est.

CONTRACT RESEARCH SUMMARY

Title: The *In Vitro* Cell and Mammary Cancer

Principal Investigator: William F. McLimans, Ph.D.
Name/Address Roswell Park Memorial Institute
Performing Buffalo, New York 14203
Organization:

Contract Number: NO1-CB-23866
Starting Date: June 15, 1972 Expiration Date: 6/14/75
Goal: To achieve satisfactory long-term cultures of human mammary tissue.

Approach: Utilizing human and animal mammary tissue, normal and abnormal. 1) Dissociation of tissue of origin into single cell suspension; determination of optimal pH and pCO_2 for mammary cells; determination of the optimal concentration of hormones in culture media under steady state conditions in collagen coated flasks. 2) Characterization of culture viability by morphology, rate of synthesis of β casein, β lactalbumin, and triglycerides. 3) EM analysis of the early events related to adaptation of cells in culture.

Progress: Fifty-three specimens of human mammary tumors have been obtained from surgery for culturing, however, a high rate of contamination was encountered. A procedure was developed which yielded a low and acceptable level of bacterial contamination. Effort was directed toward culturing Sprague-Dawley rat lactating mammary glands due to difficulty in obtaining human tumor tissue. An attempt was made to code and characterize cell types in mammary cell cultures which were classified into six different cell types. Most important an Epithelial-2(E-2) type which was detected at optimal conditions of pH 7.4-7.7, pCO_2 5-10% and in presence of prolactin and hydrocortisone was steatotic. Morphological study of the steatotic cells and culture conditions necessary to maintain them was achieved. The kinetics of steatotic cell formation, role of serum and hormones in maintaining lipid synthesis, and cloning of the steatotic cell to study the role of other cells in keeping the steatotic state will be pursued.

Significance for Cancer Research (NCP Objective 3 Approach 1)
The main objective of developing cell culture systems which retain native characteristics of the planted cells should aid in studies of the etiology of the disease which in turn should aid in prognostic and treatment studies.
Project Officer: D. Jane Taylor, Ph.D. and Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 1/21/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $50,000 est. Terminal

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigator: W. B. Dandliker, Ph.D.
Name/Address Performing Organization: Scripps Clinic and Research Foundation
La Jolla, California 92037

Contract Number:
Starting Date: Expiration Date:
Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones.

Approach: 1) Preparation of purified fluorescent derivatives of prolactin, insulin, estradiol, testosterone and growth hormone by fluorescent labeling followed by column chromotography or gel electrophoresis. 2) Preparation of cellular receptors for normal and neoplastic mammary tissue. 3) Investigation of interaction between fluorescent labeled hormones and their specific cellular receptors.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)
Investigate cellular and organismal modifiers of the transformation and promotion process in order to develop the means to prevent transformation of normal cells to cells capable of forming cancers.
Project Officer: D. Jane Taylor, Ph.D. and E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/13/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $85,202 est.

CONTRACT RESEARCH SUMMARY

Title: Documentation of Human Breast Cancer in Tissue Culture and the Recognition of Growth Patterns

Principal Investigator: Russell P. Sherwin, M.D.
Name/Address University of Southern California
Performing Los Angeles, California 90033
Organization:

Contract Number: N01-CB-23860
Starting Date: June 19, 1972 Expiration Date: 6/18/75
Goal: 1) To compare human breast cancer in tissue form with cultured monolayers by direct observation and time-lapse film recordings. 2) To establish criteria for relating H&E classification of mammary tumors to _in vivo_ and _in vitro_ behavior.
Approach: 1) Tumor tissue, sliced thin enough to be transparent, is placed in culture media and the transition of the original tissue into the culture monolayer is followed by time-lapse photos using phase contrast microscopy to study the behavior of documented cancer _in vitro_. 2) Conventional histological examination of original tumor and standard _in vitro_ culture preparations of cells thereof are carried out. 3) The pattern of outgrowth, the differential survival of various cells types, the intercellular relationships and the correlation between _in vitro_ behavior and _in vivo_ structure and clinical behavior will be studied.

Progress: Of 167 breast cancer specimens 65% yielded short term histocultures for time lapse cinematographic identification and documentation of cancer architecture. Survival of cancer in culture tended to parallel lymph node metastases, i.e., greater survival with greater node involvement. Mean survival time for histocultures was 14 days but 5 were carried for periods of 114 days to 11 months. An attempt to correlate certain forms of cytostructural patterns as discord islands, irregular islands, and non-insular cellular aggregates with lymph node metastasizing capabilities of the cancer has been carried out on 40 cases. More data will be accumulated to verify if non-insular cancer forms exhibit a marked capacity for lymph node metastases which could be of diagnostic value.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Overall objective is to conduct laboratory studies of human breast cancer _in vitro_, to correlate findings with clinical behavior of each tumor. Should provide new information on pathogenesis and prognosis for cancer in general.
Project Officer: D. Jane Taylor, Ph.D., Katherine Sanford, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/10/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $65,000 est. Terminal

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator: Frank E. Stockdale, M.D., Ph.D.
Name/Address Stanford University
Performing Stanford, California 94305
Organization:

Contract Number: N01-CB-23875
Starting Date: June 1, 1972 Expiration Date: 5/31/75
Goal: To determine the role of serum factors and hormones in the initiation and control of DNA synthesis in tissue cultures of mammary gland epithelium and mammary neoplasms of mice.
Approach: Studies include: 1) The isolation and characterization of factors in serum responsible for mammary epithelial growth; 2) Comparison of their mode of action with that of hormones which are also related to the growth of the gland; 3) Determination of the early biochemical events which precede serum factor and hormonal initiation of DNA synthesis; 4) Isolation and characterization of the DNA which is synthesized under the influence of serum factors and hormones.

Progress: A fraction has been obtained from serum which has a growth-promoting effect on mouse mammary cells 44 times that of normal serum. It was obtained using Dowex 50 x 4 and 0.1 N ammonium hydroxide elution, it is heat-stable, has a large molecular weight at neutral pH but dissociates to small size in acid conditions. Epithelium from virgin and lactating animal had a delayed response of DNA synthesis to insulin and serum; epithelium from mid-pregnancy responded immediately. There is a progressive elimination in epithelial growth response to serum as pregnancy progresses. Equal amounts of insulin are needed to induce DNA synthesis of mammary cells during pregnancy but unequal amounts of serum are necessary for the same purpose, thus, insulin cannot be the serum-promoting factor.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Elucidating factors which initiate and control DNA synthesis in normal and abnormal mammary gland tissue should provide knowledge leading to methods to prevent the development of cancer cells or control of their growth.
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 4/10/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $80,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry

Principal Investigator: Zoltan Lucas, M.D.
Name/Address Performing Organization: Leland Stanford Jr. Univeristy
Stanford, California 94305

Contract Number: N01-CB-33905
Starting Date: May 24, 1973 Expiration Date: 5/23/75
Goal: To study modifications of host's response to specific and nonspecific inhibitions of cellular immunity (ICIO) by mammary tumors.

Approach: Rat mammary tumors induced or spontaneous, and cultures of neoplastic cells to be utilized. At various intervals after tumor implantation or induction of a primary tumor the ability of the rat serum to block specific cell-mediated immunity to the tumor or to other antigens will be measured. Six aspects of the immune response to neoplasia to be measured, e.g., cytotoxic, blocking, and lymphocyte-dependent antibodies, etc. An assay to measure blocking antibodies for mammary tumors to be developed. Kinetics of specific and non-specific factors to be undertaken. Attempts to be made to demonstrate cellular immunity to mammary tumors in rodents. Pre-incubation of lymphs with membrane fractions or soluble antigens from mammary tumors to be used to inhibit cellular immunity reaction. This will constitute the basis for an assay of tumor specific antigens.

Progress: Immunogenic and metastasizing characteristics of the 13762 rat mammary tumor have been examined. These characteristics, not highly immunogenic but metastasizes widely, make it a suitable model for the human disease. Cell mediated cytotoxicity can be demonstrated in allogenic animals after i.p. inoculation by live tumor cells. Cytotoxicity can be demonstrated only in syngeneic rats after pre-immunization at least twice with irradiated cells. Cytotoxicity of multiple immunized syngeneic lymphs can be blocked by sera from animals given a single injection of viable tumor cells. The 13762 tumor can sensitize syngeneic autochthonous lymphs _in vitro_ but not _in vivo_.

Significance for Cancer Research (NCP Objective 4 Approach 1)
To determine whether non-specific or specific inhibitors of cellular immunity are directly or indirectly related to human tumors.

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/12/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $90,000 est.

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Beppino C. Giovanella, Ph.D.
Name/Address The Stehlin Foundation
Performing Houston, Texas 77025
Organization:

Contract Number: N01-CB-33856
Starting Date: February 1, 1973 Expiration Date: 1/31/75
Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential and hormone responsiveness.

Approach: A colony of thymusless-nude Swiss mice has been developed and the good health of the animals permits them to be used in long term experiments. Fifty human mammary carcinomas will be transplanted each year, each tumor to be transplanted into at least 3 females and 1 male. Tumor fragments immediately after removal will be inserted subcutaneously into two fat pads and on the back. Enrichment of the transplant with viable neoplastic cells will be attempted by preculturing the dissociated tissue - transplantation of some of these cases will be carried on. After transplantation the animals will be followed for 4 months. If the tumor grows it will be retransplanted for 3 generations as feasible. The transplanted tumor fragment will be characterized by a pathologist. The morphology and karyotype of the growing tumor will be compared with the original fragment.

Progress: Twenty-six primary and two metastatic human breast cancers have been transplanted into female and male athymic mice. Fifteen of the primaries and one of the metastatic ones have grown at the site of subcutaneous inoculation at the time of this report, growth is as slowly enlarging nodules appearing 30 to 150 days after implant. Two primaries grew explosively after a latent period of 5 months, rapidly killing 3 animals. Five human breast cancer-derived cell lines have also been injected subcutaneously at a dose of $1x10^7$ cells. Two have already given origin to malignant invasive tumors. The athymic mouse colony in this laboratory is probably the best in the world and the life span of these mice is definitely superior to those of any other laboratory in the world.

Significance for Cancer Research (NCP Objective 5 Approach 6)
The continued growth of human cancer cells of any origin in an animal would enable prognostic studies for treatment of the disease on an indivudual patient basis.
Project Officer: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 10/27/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74Funds: $98,500

CONTRACT RESEARCH SUMMARY

Title: Growth Alteration of Mammary Neoplastic Cells Obtained by Manipulation of Cellular Environment

Principal Investigator: Creed W. Abell, Ph.D.
Name/Address University of Texas Medical Branch
Performing Galveston, Texas 77550
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To manipulate cellular environment *in vivo* and *in vitro* in order to limit preferentially the growth of neoplastic cells of mammary origin.

Approach: 1) Additional purification of phenylalanine ammonia lyase (PAL) from *Rhodotorula glutinis*. 2) To evaluate the effects of PAL from *Rhodotorula* and phenylalanine restriction on human breast cancer cells *in vitro*. 3) To evaluate the effects of PAL of the same source on human breast cancer in athymic mice, in mice bearing the BW 10232 mammary adenocarcinoma and in C_3H mice with spontaneous mammary tumors. 4) Isolate PAL from *Ustilago hordei* which specifically deaminates phenylalanine and not tyrosine (PAL from *Rhodotorula* deaminates both) and test as in (2) and (3) above.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 4)
Develop the means to cure cancer and to retard the progress of cancer not cured through enhancement of the host's ability to eliminate or prevent development of cancer.
Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 3/14/74
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $89,624 est.

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine Induced Breast Cancer Regression

Principal Investigator: William L. McGuire, M.D.
Name Address University of Texas Medical School
Performing San Antonio, Texas 78229
Organization:

Contract Number: N01-CB-23862
Starting Date: June 1, 1972 Expiration Date: 5/31/75

Goal: To study the relationship between estrogen binding protein (EBP) and hormone dependency of mammary tumors.

Approach: Human mammary carcinomas and metastasis thereof; also primary, 7,12-DMBA induced, and transplantable R3230AC, MT/W9A, 13762E, 13762NF, 13762TP, 3M2N, rat mammary tumors will be studied in the following manner: 1) Quantitation of EBP in 8 rat mammary tumors and correlation to hormone dependency of each tumor. 2) Capacity of isolated chromatins to bind estradiol + EBP from exogenous cytosol. (This test is expected to show whether a "defect" of the chromatin acidic proteins may cause poor binding capacity and therefore hormone independence). 3) Search for correlation between loss of hormone dependency and chromatin acceptor activity during changes occurring in serial transplantation of rat tumors. 4) Quantitation of EBP absolute level in human mammary tumors and their metastasis. Correlation with their future behavior.

Progress: Considerable attention has been given to improving and extending methodology, recent developments are: lability of receptor proteins during determination may be a severe source of error; an assay for solid phase ligand exchange of cytoplasmic, estrogen bound and nuclear compartments measured; determination of absolute estrogen receptor (ER) amounts. Correlations between estrogen receptors and objective remission of patients are becoming available. In the rat mammary tumor studies it has been found that nuclei of unresponsive tumors bind as much ER as nuclei of target tissue. Unresponsiveness depends on lack of cytoplasmic receptor. Nuclei of tissue such as stomach and liver (unresponsive) also bind large amounts of ER. A large number of acceptor sites for ER exist but are far from being saturated *in vivo*. *In vitro* binding of ^{125}I prolactin to lactating gland and the R3230-AC tumor of rats has been found. Determinations of prolactin and androgen receptors have been undertaken and studies are underway to determine their quantitative relation to tumor regression. A transplantable tumor subline (MTW9-MD) has been developed which requires only physiological prolactin levels and regresses after ovariectomy.

Significance for Cancer Research (NCP Objective 5 Approach 6)
Assessment of estrogen binding sites for each human mammary tumor could become a routine clinical procedure for predicting therapy for the individual patient.

Project Officer: D. Jane Taylor, Ph.D., E. Bradford Thompson, M.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 2/24/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $127,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3',5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator:	David Maudsley, Ph.D.
Name/Address Performing Organization:	Worcester Foundation for Experimental Biology Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33919
Starting Date: June 1, 1973 Expiration Date: 5/31/75
Goal: To study the role of cyclic adenosine 3', 5'-monophosphate (cAMP) in the developing mouse mammary gland and to establish whether the incidence of mammary tumors alters cAMP metabolism.
Approach: Separation of epithelial from adipose cells from virgin, pregnant, and lactating mammary tissues of C57 and C3H mice to be accomplished using collagenase procedure. Subcellular fractionation procedures to be applied for the localization of adenyl cyclase, cAMP receptor protein and phosphodiesterase, relative amounts determined. cAMP determined by protein binding assay using bovine adrenal protein free from hormonal nucleotides, separated by BSA-coated charcoal. Phosphodiesterase and adenylcyclase determinations to be based on the specificity of cAMP binding protein. Formation of cAMP of intact cells studied by preincubating tissue slices or cells with prelabeled precursors. Isolation of cAMP and related nucleotides to follow using paper chromatography. Several major components of cAMP as an integrated unit will be studied in isolation and an attempt will be made to establish the relative significance of each within the intact cell and then in the whole gland.

Progress: Cell suspensions of mammary tissue have been prepared. Cells show response to epinephrine and prostaglandins E_1 and E_2 but no response as far as cAMP when prolactin, insulin or glucocorticoids are added. The response appears to be related to epithelial cells, however fibroblast contamination must be considered. Tumor cells appear to be equally responsive to the hormones as are the normal cells preparations. cAMP level changes during pregnancy and lactation, parallel changes in the amount of assayed adenylcyclase.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Develop the means to prevent transformation of normal cells through investigation of cellular and organismal modifiers of the transformation and promotion process.
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology Site Visit Date: 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY 74 Funds: $70,000 est.

CONTRACT RESEARCH SUMMARY

Title: Studies on Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Name/Address Performing Organization:	**Sumner Burstein, Ph.D.** Worcester Foundation for Experimental Biology Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-33920
Starting Date: June 1, 1973   Expiration Date: 5/31/75
Goal: To measure the levels of prostaglandin (PG) biosynthesis in developing adult neoplastic mammary tissue.

Approach: Intracellular PG synthesis will be measured by incubation of the enzyme preparations with 8,11,14 eicosatrienoic and arachidonic acid under optimal conditions already established for preparation from seminal vesicles. The incubation mixture will be acidified, extracted with chloroform-methanol, treated with alkali, chromatographed in silica gel and counted. The enzyme preparation will consist of microsomal fractions from homogenates of the whole gland. Several parameters such as effects of glutathione, epinephrine or various inhibitors will be tested and compared with data from other tissues such as seminal vesicle which the author has studied. If microsomal fractions prove to be devoid of PG synthetase systems, whole cell cultures will be used also. Dr. Fairbanks will supervise the operation. Tissue sources will be C57BL, C3H, C3Hf, GR/A. The PG synthetase systems will be studied in developing adult, pregnant, and lactating neoplastic tissue. If any difference is found, the effects *in vivo* and *in vitro* of PG inhibitors will be tested. Radioimmunoassays for PG metabolites are currently being developed. Monitoring of their levels in relation to PG synthetases will be pursued although detailed protocols cannot yet be drawn.

Progress: Considerable data have been obtained in studies of PG biosynthesis of mouse mammary microsomes, however, they show little or no evidence of activity. Because of these results a natural inhibitor in mammary tissue has been postulated. The microsomes problem turned attention to mammary cell suspensions and primary cultures for biosynthetic studies of PG, the results to date have been encouraging. Studies of blood levels of PGs (PGE, F and F metabolites) in mice have been initiated using three radioimmunoassays, it is too early to draw conclusions.

Significance for Cancer Research (NCP Objective 3 Approach 3)
Develop the means to prevent transformation of normal cells to cells capable of forming cancer through investigations of cellular and organismal modifiers of the transformation and promotion process.
Project Officer: D. Jane Taylor, Ph.D., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology   Site Visit Date 5/3/73
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $40,000 est.

CONTRACT RESEARCH SUMMARY

Title: Intracellular Membrane Biogenesis in Hormone-Medicated Differentiation of Mouse Mammary Epithelium *In Vitro*

Principal Investigator: Grant Fairbanks, Ph.D.
Name/Address Worcester Foundation for Experimental
Performing Biology
Organization: Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-23874
Starting Date: June 16, 1972 **Expiration Date:** 6/15/75

Goal: To investigate the role of epithelial cell membranes in mouse mammary gland differentiation and tumorigenesis.

Approach: Using virgin, pregnant-lactating, involuting mammary gland of five mouse strains and primary cultures of epithelial cells of the same glands, carry out the following studies: electron microscopic characterization of endoplasmic reticulum, golgi and plasma membrane preparations of mammary gland during differentiation and after *in vivo* action of viral, genetic and hormonal factors on plasma membrane; search for aberrant membrane states associated with "dome" system as a way of transferring cells in vitro and study the plasma membrane during cell differentiation and response to oncogenic agents.

Progress: Considerable investigation into methods of cell dissociation have been carried out. Short-term monolayer cultures of normal epithelial and mammary carcinoma cells have been established. The role of insulin and hydrocortisone in "dome" formation was assessed. Preliminary polypeptide characterization of microsomal fractions were begun. Subcellular fractionation of mammary epithelium has been accomplished. Considerable cross-contamination has been experienced as determined by the presence of marker enzymes. Polypeptide composition of microsomal membranes was examined by electrophoresis. During lactation rough membranes demonstrate increase in 55,000 and 75,000 dalton polypeptides and the appearance of a new 59,000 dalton component. A new 80,000 dalton component appears in smooth membranes during lactation. NADH-cytochrome C reductase activity is associated with smooth membranes during pregnancy and with the rough endoplasmic reticulum after the first week of lactation. An improved polychrome staining method for thick epoxy sections has been developed.

Significance for Cancer Research (NCP Objective 3 Approach 5)
Studies of epithelial cell membranes should contribute new information leading to a better understanding of the mechanism of tumor growth and regression.

Project Officer: D. Jane Taylor, Ph.D., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology **Site Visit Date:** 4/18/72
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds: $65,000 est. Terminal

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Lewis M. Schiffer, M.D.
Name/Address Allegheny General Hospital
Performing 320 East North Avenue
Organization: Pittsburgh, Pennsylvania 15212

Contract Number: N01-CB-43899
Starting Date: February 1, 1974 Expiration Date: 1/31/75
Goal: To develop an approach to cell kinetics which determines growth fractions and cell cycle times of human breast cancer tissue and is potentially adaptable to the study of the effects of various forms of treatment upon these factors.

Approach: The project will use surgical and biopsy specimens of human breast cancer tissue, either primary or metastatic. Some patients with metastatic cancer will receive tritiated thymidine after an initial biopsy in order to compare in vivo and in vitro labelling techniques. Growth fraction, cell cycle time, and turnover rates of the tumor tissues will also be studied by analyses of the enzymes DNA primer-dependent DNA polymerase (PDP) and DNA-dependent DNA polymerase (DDDP). When these techniques are reliable, it is planned to perform similar studies on patients after standard dosages of radiotherapy or anti-cancer drugs.

Progress: The adaptation of the in vitro methods is underway, using 13762 rat tumor.

Significance for Cancer Research (NCP Objective 5 Approach 6)
(and Objective 6, Approaches 1, 2, 5, and 6)

Project Officer: Dr. Richard Simon, and Dr. Douglass Tormey
Program: Breast Cancer Treatment Site Visit Date: 9/10/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II and Stage III Carcinoma of the Breast

Principal Investigator: Dr. Frank C. Sparks
Name/Address University of California at Los Angeles
Performing Los Angeles, California
Organization:

Contract Number:
Starting Date: June 20, 1974 Expiration Date 6/20/75
Goal: To determine the relative effectiveness of adding BCG alone or chemotherapy and BCG to mastectomy in patients with locally advanced tumor.

Approach: Patients with any number of positive axillary lymph nodes will be eligible for this study. After mastectomy patients will be randomly assigned to one of three regimens: 1) observation only (control), 2) BCG immunotherapy or 3) BCG plus cyclophosphamide, methotexate and 5-flourouracil. The cycles will be repeated for one year. All patients will be observed regularly for evidence of treatment failure.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date:4/3/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $210,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II and Stage III Carcinoma of the Breast

Principal Investigator: Dr. Charles Hubay
Name/Address Performing Organization:
Case Western Reserve University
University Hospitals of Cleveland
2040 Adelbert Road
Cleveland, Ohio 44106

Contract Number:
Starting Date: June 15, 1974 Expiration Date: 7/15/75

Goal: To test the effects of adding chemotherapy, anti-estrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer.

Approach: Patients who have four or more axillary nodes involved with metastases at the time of surgery will be eligible for this study. Stratification, but not treatment selection, will be on the basis of the presence or absence of estrogen receptor protein in the tumor. Random treatment assignments shall be 1) observation only (control), 2) cyclophosphamide, methotrexate 5-fluorouracil, nafoxidine (CMFN) for 6 months or 3) CMFN for 6 months plus BCG for 6 months. Endpoint will be the first evidence of treatment failure i.e. the appearance of local recurrent or distant tumor.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date: 4/1/74
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $150,000

CONTRACT RESEARCH SUMMARY

Title: Develop a Method for Predicting Response to Adrenalectomy

Principal Investigator: Name/Address Performing Organization:	Dr. Elwood Jensen University of Chicago Chicago, Illinois

Contract Number: N01-CB-60945
Starting Date: June 16, 1966 Expiration Date: 6/15/75
Goal:

Approach: Breast Cancer has long been considered either "hormone-dependent" or autonomous, but any clinical test for such a distinction has been lacking. Since 1967 Dr. Jensen has been studying estrogen receptors and more recently has been examining the relationship between estrophile level and response to endocrine therapy. Patients who have had receptor assays performed on their primary and/or metastatic tumors undergo appropriate additive or ablative endocrine therapy. In most instances the choice of therapy is not dependent upon assay results. The data will be analyzed for correlation between clinical response and estrophile receptor levels. Dr. Jensen is also working on methodology for other receptor protein determinations and on modifications of estrophile assay method.

Progress: 115 patients have been entered on the study outlined above. Extramural case review, using standard cooperative group procedures and criteria, has been performed on about half of the patients in the study. Additional tumor specimens have been examined for the presence of receptors. Most of these have been primary breast cancers and the data from these will be available to correlate with the patients' clinical course.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Erwin P. Vollmer, Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date: 10/23/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $79,000

CONTRACT RESEARCH SUMMARY

Title: Study of Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: **Dr. Charles Vogel**
Name/Address Performing Organization: Emory University School of Medicine
Atlanta, Georgia

Contract Number:
Starting Date: June 30, 1974 Expiration Date: 6/30/75

Goal: To determine whether the incidence and/or duration of anti-tumor effectiveness are increased by combining hormone therapy and chemotherapy.

Approach: Postmenopausal women with their first evidence of breast cancer are assigned to one of two treatment regimens: 1) Estrogens or 2) Estrogens plus cyclophosphamide, adriamycin, and 5 fluorouracil. With documented failure of estrogen therapy, chemotherapy will be initiated. The patients will be observed for objective evidence of tumor regression, duration of remission, and quality of performance status.

Progress: New Contract

Significance for Cancer Research (NCP) Objective 6 Approach 1)

Project Officer: Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date:
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $87,000

CONTRACT RESEARCH SUMMARY

Title: Investigation of Steroid Sulfational Estrogen-Binding in Human Breast Cancer

Principal Investigator: Dr. Thomas Dao
Name/Address
Performing
Organization: Health Research, Inc., Roswell Park Division
and New York State Department of Health
666 Elm Street
Buffalo, New York 14203

Contract Number: N01-CB-43900
Starting Date: February 1, 1974 Expiration Date: 1/31/75

Goal: To determine the relationship of steroid sulfotransferase activity and of estrophile receptor levels to tumor response to therapy and to risk of tumor recurrence.

Approach: The above biochemical studies will be performed on human breast cancer tissue obtained at the time of mastectomy. Between 200 and 300 patients will be studied. Those who show clinical evidence of treatment failure (tumor recurrence) will undergo the appropriate endocrine ablation therapy and the clinical responses correlated with the biochemical parameters of the tumors.

Progress: Dr. Dao's research associate visited Dr. McGuire's laboratories to assure standardization of estrophile receptor assay techniques. The necessary laboratory equipment has been assembled. Clinician-collaborators are prepared to submit tissues to the study. No data from this study are as yet available.

Significance for Cancer Research (NCP Objective 6 Approach 6,4)

Project Officer: Dr. Mary E. Sears, Dr. Paul Carbone
Program: Breast Cancer Treatment Site Visit Date: 6/5/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $152,000

CONTRACT RESEARCH SUMMARY

Title: Studies in Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: **Dr. Arthur E. Bogden**
Name/Address **Mason Research Institute**
Performing **Worcester, Massachusetts 01608**
Organization:

Contract Number: NO1-CB-43914
Starting Date: Nov. 1, 1965 Expiration Date: 10/31/75

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities (surgery, chemotherapy, immunotherapy) in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in four transplanted rat mammary tumors. Two mammary tumors will be used for evaluating: 1) some compounds known to affect serum prolactin levels of the hosts, e.g., ergot derivatives, L-Dopa, etc.; 2) 10 to 12 agents of known clinical effectiveness in some breast cancers as suggested by the Breast Cancer Treatment Committee, e.g., Cytoxan, Methotrexate, 5-Fluorouracil, Thiotepa, etc.; 3) antiviral agents. Compounds will be tested for activity in inhibiting early phases of tumor growth, as well as for inducing regression of the 13762 tumor which metastasized routinely to the lungs and other organs of the host and their effects on host hormone levels, e.g., prolactin, FSH, LH, etc. A small amount of work continues on immunotherapy with a methanol-soluble fraction of Mycobacterium butyricum MSF-MB as a non-specific immune stimulating agent.

Progress: Twelve agents known to have had some clinical effectiveness in breast cancer have been tested in three transplantable rat mammary tumors. The 13762 tumor is the most responsive to all agents while the R-35 is least responsive. Adriamycin, phenylalanine mustard, and cyclophosphamide were the most effective of the agents in inhibition of tumor growth in all three systems. Two drug combinations are now being evaluated in the13762 system, e.g., Adriamycin as the primary drug is being tested at 3 dose levels and the ancillary agents, Cytoxan, 5-Fluorouracil, Methotrexate, etc., at two dose levels. Adriamycin and Cytoxan also are being tested in the established tumor assay; the combination of agents does not appear to be more oncolytic than Cytoxan alone and toxicity is greater than either alone. A paper on combination therapy (surgery, chemotherapy and immunotherapy) in the 13762 tumor system will appear in Cancer Research in the summer of 1974.

Significance for Cancer Research (NCP Objective 6C Approach 1)
Evaluate methodologies of immunology, chemotherapy, surgery, and irradiation (alone and in combinations) in experimental mammary tumor systems which could contribute to information for treatment of human breast cancer.
Project Officer: D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment Site Visit Date: 3/22/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: An Evaluation of Surgical Adjuvant Chemotherapy Utilizing 5-Fluorouracil, Cytoxan and Prednisone in Patients with Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Dr. David L. Ahmann Mayo Clinic Rochester, Minnesota

Contract Number: N01-CB-33899
Starting Date: June 30, 1973 Expiration Date: 6/29/75

Goal: To assess the effects of surgical adjuvant chemotherapy, utilizing a multiple drug program in patients with locally advanced mammary carcinoma.

Approach: Patients with unfavorable primary breast cancers will be randomly assigned to treatment programs involving surgery alone, surgery followed by chemotherapy, surgery followed by radiation therapy and surgery followed by radiation therapy and chemotherapy. High risk patients with one or more positive axillary nodes will have surgery including complete removal of the breast and axillary contents with preservation of the pectoral muscles if possible. After suitable stratification (according to menstrual category, tumor size, degree of nodal involvement) patients will be randomly assigned to treatment groups. Adjuvant chemotherapy will be initiated two weeks post-operatively (during radiation when this also is given): 5-Fluorouracil and orally for 5 days. The chemotherapy will be repeated every six weeks until evidence of recurrent or metastatic disease occurs or for one year, whichever occurs first.

Progress: Patient entry into the study began in September, 1973, and case accrual is at the rate of about 6 per month. Follow-up data are available upon all patients; no conclusions can as yet be drawn about the relative effectiveness of the above therapeutic regimens.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Paul Carbone
Program: Breast Cancer Treatment Site Visit Date:
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $95,000 est.

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Group (NSABP)

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing Pittsburgh, Pennsylvania
Organization:

Contract Number: N01-CB-23876
Starting Date: June 30, 1972 Expiration Date: 6/29/76
Goal: 1) To determine the relative merits of total (simple) mastectomy and radical mastectomy and 2) to evaluate the efficacy of prolonged chemotherapy after mastectomy.

Approach: In Protocol IV, patients with operable cancer of the breast are designated as having clinically positive or clinically negative axillary nodes and then randomly assigned to a treatment program: A. With positive nodes (1) radical mastectomy or (2) total mastectomy with regional radiation; B. With negative nodes (1) radical mastectomy (2) total mastectomy or (3) total mastectomy with regional radiation. In Protocol V, the chemotherapy study patients with one or more metastatic axillary nodes will undergo conventional radical or modified radical mastectomy followed by randomly assigned 1) L-Phenylalanine Mustard-0.15 mg/Kg/day p.o. x 5 days every 6 weeks or 2) placebo in equivalent dosage form. Chemotherapy begins 2-4 weeks after mastectomy and continues for 2 years or until treatment failure is recognized, whichever comes first.

Progress: Thirty-three institutions are actively participating in the mastectomy study and 35 institutions in the chemotherapy study. Patient accrual has proceeded well and the projected 1600 entries are almost complete for the surgical study and the planned 300 patients for the chemotherapy study will probably be entered before January, 1975. The studies have not been in progress sufficiently long to provide significant data concerning relative treatment failure or patient survival. Follow-up data collection is operating at a 98+% level assuring that the studies will provide information concerning epidemiologic and prognostic factors as well as treatment effects.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Paul Carbone, Dr. Alfred Ketcham
Program: Breast Cancer Treatment Site Visit Date: 12/14/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $500,000

CONTRACT RESEARCH SUMMARY

Title: New Techniques in Cell Kinetics of Breast Cancer

Principal Investigator: Robert M. Zucker, Ph.D.
Name/Address Papanicolaou Cancer Research Institute
Performing 1155 N. W. 14th Street
Organization: Miami, Florida 33136

Contract Number: N01-CB-33861
Starting Date: June 27, 1973 Expiration Date: 2/26/75
Goal: The goal of this project is to develop an approach to cell kinetics which is potentially applicable to the study of patients with breast cancer.

Approach: For these studies, a series of rat mammary carcinomas will be the experimental model. Cells from tumor slices will be dispersed enzymatically and the dissociated cells washed in tissue culture medium at 4°C, and subjected to buoyant density separation or velocity sedimentation. The separated cells will be characterized as to cell cycle times and growth fractions by several procedures, including autoradiography, microspectrofluorometry, immunofluorometry, and electron microscopy. When preliminary studies have demonstrated that cell cycle measurements are reliable, similar studies will be made with tumors and animals subsequent to injections of chemotherapeutic agents and hormonal agents.

Progress: Single viable C_3H mammary tumor cells have been enriched using a combination of mechanical and enzymatic techniques. Centrifugal cytology and autoradiographic methods are yielding good morphology of labeled single dispersed CHO cells and measurements of mitotic index of dividing cells, Application of these methods to rat metastasizing mammary tumor is under study.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Douglass Tormey, Dr. Erwin P. Vollmer
Program: Breast Cancer Treatment Site Visit Date: 3/14/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY73 Funds: $183,550

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator:
Name/Address
Performing
Organization:

Dr. Joseph Post
Goldwater Memorial Hospital (NYU)
New York, New York

Contract Number: N01-CB-33903
Starting Date: June 28, 1973 Expiration Date:6/27/75
Goal: To provide information concerning cell cycle parameters in patients with breast cancer.

Approach: *In vivo* labeling with tritiated thymidine will be coupled with *in vitro* labeling C^{14}-thymidine. This is carried out by intravenous injection and local injection of HTdR into selected patients with advanced breast cancer and subsequent study of tumor tissue samples from these patients by cell culture, cell dispersion and *in vitro* labeling techniques. The measurement of effect of anti-cancer treatments upon the proliferating cell pool is one of the ultimate objectives. This would permit evaluation of therapeutic effectiveness long before clinical observation of change in tumor size. New mathematical models are being designed by Dr. Post and Dr. Simon; these will be utilized for analysis of the data.

Progress: Tumors have been successfully cultured and the labeling methodologies have been applied to the cultured cells. A promising cell separation technique has also been developed. However, there have been delays due to withdrawal of institutional consent for administration of ^{3}HTdR to human subjects and to temporary unavailability of autoradiographic emulsion. It is anticipated that consent will soon be obtained from other potential collaborating institutions and that the *in vivo* studies can proceed.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Douglass Tormey, Dr. Richard Simon
Program: Breast Cancer Treatment Site Visit Date:3/9/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. H. H. Sky-Peck
Name/Address Rush-Presbyterian-St. Luke's Medical
Performing Center
Organization: Chicago, Illinois

Contract Number: N01-CB-33886
Starting Date: June 30, 1973 Expiration Date: 6/29/75
Goal: The goal of this contract is to provide estimates of cell cycle time parameters, and to relate these parameters to therapeutic results.

Approach: The approach which will be used will be that of utilizing tritiated thymidine _in vivo_ pulses with C^{14}-thymidine _in vitro_ pulses followed by autoradiographic analysis. Clinical coordination will be done retrospectively by comparing the cell kinetic data with the therapeutic modalities employed and the results of treatment. The dividing cell pool, generation time, and duration of cell cycle time is to be determined in breast cancer tissue from about 40 patients. These data will be determined for both primary and metastatic lesions, for the tumor center, mid-zone, and periphery, and for large and small tumor masses.

Progress: Since August, 1973, thirty-seven patients have been entered into the study and cell kinetic data are available on about half. The adequate dose of tritiated thymidine for radioautography has been established as 10mCi. Preliminary comparisons are being made of DNA synthesis with the presence or absence of estrogen-receptor. _In vitro_ labeling of tissues obtained from various areas of tumor masses shows the greatest cellular activity toward the periphery. Information is yet to be obtained on the lengths of various phases of cell cycles and the various responses from anti-cancer therapy. This is the current area of emphasis for the study.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Douglass Tormey, Dr. Richard Simon
Program: Breast Cancer Treatment Site Visit Date: 5/9/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $45,000 est.

CONTRACT RESEARCH SUMMARY

Title: Study Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Dr. Charles Perlia
Name/Address Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
1735 West Congress Parkway
Chicago, Illinois 60612

Contract Number:
Starting Date: June 30, 1974 Expiration Date:6/30/75

Goal: To assess the effect of adding chemotherapy to oophorectomy in the treatment of premenopausal women with breast cancer.

Approach: At the first evidence of failure of treatment of primary breast cancer, surgical castration shall be performed on premenopausal patients. When evaluation is carried out 12 weeks after castration, anti-tumor effect will be classified as 1) Failure, 2) No Change, 3) Regression. Those showing no change or tumor regression shall be randomly assigned to 1) observation or 2) cyclophosphamide, 5-flourouracil, methotexate (CMF). When relapse occurs in the patients assigned to observation, CMF will be started. This will allow comparison of the effectiveness of concomitant and sequential therapy.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date:
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $65,000

CONTRACT RESEARCH SUMMARY

Title: Prolactin Radioimmunoassays in Human Serum

Principal Investigator: Name/Address Performing Organization:	Dr. W. P. VanderLaan Scripps Clinic and Research Foundation LaJolla, California

Contract Number: NO1-CB-33881
Starting Date: June 29, 1973 Expiration Date: 6/28/75
Goal: To provide 1000 radioimunoassays of prolactin in human serum per month in collaborative research on breast cancer.

Approach: Human prolactin (h PRL) has been used to produce antibodies in rabbits by techniques of Goudie et al and for radioiodination (125_I) construction of standard curves. Antibody on hand is suitable for assay purposes in initial dilutions of 1:20,000 and 1:40,000. Sera will be assayed in duplicate and assays will be run at two to four weekly intervals according to volume of work. Protocols for research will be agreed upon by the Principal Investigator, cooperating clinicians, the Project Officer and a representative of the Treatment Committee.

Progress: The Medical Breast Service of NCI has submitted approximately 200 blood samples from patients with breast cancer. Arrangements are underway for collaboration with Dr. Henry Lynch for prolactin level comparison studies on breast cancer patients, their family members and controls, and with Dr. T. C. Hall for clinical studies involving L-DOPA therapy.
Dr. VanderLaan has designed physiologic studies in human volunteers (water-loading, TSH provocation, and L-DOPA inhibition) which have been approved by his institution and by the Treatment Committee and are under consideration by the NIH Clinical Center Medical Review Board.

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Dr. Erwin P. Vollmer, Dr. Mary E. Sears
Program: Breast Cancer Treatment Site Visit Date: 4/2/73
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY74 Funds: $100,000 est.

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator:	**Dr. Federico Welsch**
Name/Address	Worcester Foundation for Experimental
Performing	**Biology**
Organization:	Shrewsbury, Massachusetts 01545

Contract Number: N01-CB-43867

Starting Date: August 1, 1973 Expiration Date: 10/15/75

Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: The contractor will carry out determinations of estrophilic protein levels on tumor material of patients provided by collaborating clinicians. Both the sucrose gradient method and the charcoal methods will be used, and methodology will be constantly improved. In terms of RFP-72-17, this is a type I institution -- one which will carry out approximately 500 sample determinations per year and is not obliged to supply patients for the study.

Progress: Approximately 100 tumor samples have been assayed, the majority submitted from the Primary Breast Cancer Therapy Group under Dr. Bernard Fisher's direction. Other potential clinician-collaborators have been visited by members of the Treatment Committee's Hormone Studies Group. Dr. T.C. Hall of F.S.C. is preparing a protocol. The Treatment Committee has approved expansion of the study to include steroid sulfurylation determinations on the tumor samples of sufficient size. One obstacle to specimen submission was met by allocating funds to reimburse the clinical investigators for tissue collection expenses.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Project Officer: Dr. E.P. Vollmer, Dr. M.E. Sears, and Dr. I.J. Masnyk

Program: Breast Cancer Treatment Committee Site Visit Date: 6/7/73

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY74 Funds: $60,000

ANNUAL REPORT SUMMARY

CANCER DIAGNOSIS CONTRACT PROGRAM
July 1, 1973 to June 30, 1974

Since its inception in the summer and fall of 1972 the Diagnosis Branch of the Division of Cancer Biology and Diagnosis has expanded rapidly. Much of the energy of the Branch and the Division early in its organization was devoted to the earlier detection of breast cancer and the 27 Breast Cancer Demonstration Projects which are funded through the Division of Cancer Control. During this past year, most of the 20 of the original projects became operational and it is expected that all of them will be operational by June 30, 1974. Seven more (the remainder of the approved projects) have been funded and will be negotiated within the next few months and will be operational by the end of this calendar year. About 35,000 patients will have been screened by June 30, 1974. Biopsies are recommended at an average rate of 4 percent and of these about 25 percent are being reported as cancer. Most significantly, the incidence of node involvement in these patients ranges between 10 percent and 40 percent, well below the expected 60 to 70 percent. As an adjunct to these projects we are running a study for the comparison of the value of thermography vs mammography. This study is being conducted at Jefferson Medical School and at the Guttman Institute. Results are not yet available.

In the past year three lung cancer projects have been negotiated with the Mayo Foundation, Johns Hopkins University Medical School and Memorial Hospital-Strang Clinic in New York City. Each of these projects will screen at least 5,000 persons, who are heavy smokers, annually for lung cancer with frequent sputum cytology and chest x-ray examinations. Localization of lesions is accomplished with fiberoptic bronchoscopy. The goal is to detect lung cancer at an early enough stage so that it can be removed and cured by a surgical procedure that will be less than a pneumonectomy. Forty-five hundred persons have already been screened at the Mayo Clinic, and about 450 persons at the Johns Hopkins Medical School. Memorial Hospital is just starting to screen. The University of Cincinnati Statistical Center has been selected to coordinate the operation of these three cooperating groups. At Johns Hopkins University Medical School studies continue on the use of tantalum powder in the localization of lung tumors, cytogenetics of lung cancer cells in sputum, immunological procedures in the detection of lung cancer and other procedures. The Mayo Clinic is studying the role of Aryl Hydrocarbon Hydroxylase in heavy smokers and persons with lung cancer. At Memorial Hospital in New york City, studies of a squamous cell antigen will be related to lung cancer. Work on a tantalum delivery system for localization of lung tumors has been completed by Dr. M. Corn at the University of Pittsburgh, but clinical and other studies of tantalum powder will continue at New York University under Dr. Lippmann and at Johns Hopkins University Medical School under Dr. Stitik.

The Diagnostic Radiology Committee has devoted much time to the study and consideration of improved x-ray imaging of the chest and other areas of the body. To date none of the new imaging methods, such as subtraction techniques, etc., have seemed sufficiently promising to warrant support. There are theoretical methods now proposed that will be given much consideration and contracts may well be negotiated for this in fiscal year 1975. A contract will be negotiated with Hahnemann Medical School for the evaluation of a new method of x-ray imaging proposed and patented by Xonics Corporation.

Upon the advice of both review committees, contracts will be awarded to five institutions, Massachusetts General Hospital, Columbia Presbyterian Medical Center, the Mayo Clinic, George Washington University Medical School and the Cornell University Medical School for the evaluation of computer assisted tomography of the brain, a non-invasive technique believed to be of great promise in the field of neuroradiologic diagnoses. It is anticipated that this new technique may eventually displace the use of radio-opaque materials or air injection, both of which produce considerable morbidity, in the diagnosis of intracranial lesions.

At the Mayo Clinic, work will continue on the study of carcinoembryonic antigen in conjunction with other tests for cancer to determine if any of these combinations will assist in the earlier diagnosis of gastrointestinal cancer.

Contracts will be awarded in FY 1974 to three cooperating institutions, Mayo Foundation, the University of Chicago and Memorial Hospital-Sloan Kettering Institute, who will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

A contract will be awarded to Duke University Medical School for the feasibility study of establishing a registry of patients with cancer who have had intensive cancer chemotherapy and/or radiation therapy for cancer and have survived five years or more with no evidence of recurrent disease. These patients are at high risk for second types of cancer, other disease states related to their therapy (i.e., myxedema, adhesive pericarditis, etc.) and also provide an ideal population in which to follow the sequential levels of substances believed to be related to cancer such as CEA or alpha fetoprotein. Such a registry will be nationwide and should be maintained for at least 30 years or as long as such patients survive.

Three contracts will be awarded (to Baylor University Medical School, State University of New York Medical School at Brooklyn "Downstate" and Johns Hopkins University Medical School) for the Study of Nuclear Magnetic Resonance in normal, non-neoplastic diseased tissue and neoplastic tissue. Certain physical signals called spin lattice (T_1) and spin-spin (T_2) relaxation times have been found to be different in cancer tissue as compared to normal tissue. This contract is for encouragement of more study in this area and more evidence that the signals from cancer tissue are specific and not the result of granulomatus disease, necrosis or hemorrhage. Properly blinded studies, assuring objectivity, are planned which should establish the validity of this technique.

Two contracts (to Stanford University and to the University of Buffalo-Roswell Park) will be awarded for the detection of biochemical markers, such as a specific acid phosphatase, which may indicate a specific type of cancer or any type of cancer. One contract (to Harbor General Hospital in Los Angeles) will be awarded for the development of hormone markers in the early diagnosis of cancer.

Two contracts (to Mt. Sinai Medical School and to the University of Louisville) will be awarded to contractors who will develop a quick sensitive method for the detection of human blood in stool specimens, and if such a method is forthcoming, it will be applied to humans in a screening program to be developed.

A contract for a silicone type enema that will complement fiberoptic coloroscopy in the detection of colonic cancer is still being sought.

Two contracts for the better preparation of the bowel prior to fiberoptic colonoscopy and barium enema will be awarded to the American College of Radiology and to the University of Rochester.

Contracts to institutions with proper personnel and equipment to produce a proton beam which will critically determine differences in tissue density will be awarded to the University of Chicago and to the University of California at Berkeley.

Discussions have considered a number of projects which may be advertised and possibly selected for fiscal 1975. Among those now being considered are:

1. Screening by means of occult blood determination a large number of persons yearly for GI tract cancer. (Until a satisfactory method for the detection of human blood in stool is found, the usual method will be used in this project.)

2. Studies to develop mathematical models for the proper interval for screening by cancer site, method of screening, etc. (i.e. to develop a screening plan, etc.).

3. Multi-site screening for cancer and its feasibility.

4. Urinary cytology and determination of urinary CEA in the detection of bladder cancer.

5. Improved methods of x-ray imaging by elimination of scatter while retaining resolution factors.

6. Use of self-obtained vaginal smears in the detection of uterine cancer.

During most of fiscal 1973 Dr. Kenneth B. Olson was Chief of the Diagnostic Branch, but on December 1, 1973 Dr. Olson resigned and was succeeded by Dr. William Pomerance who has continued to supervise those projects developed in FY 1973 and FY 1974, has continued the search for awardees for FY 1974 projects and has developed the new projects proposed for FY 1975.

CONTRACT RESEARCH SUMMARY

Title: Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Arthur J. Present, M.D.
Name/Address Arizona Medical Center, Univ. of Arizona
Performing Tuscon, Arizona 85724
Organization: American College of Radiology, 6900 Wisconsin Ave.
Washington, D. C. 20015

Contract Number:
Starting Date: Expiration Date:

Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24 hr period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: Under the administrative supervision of the American College of Radiology the comparative efficiency of 8 selected colon cleansing regimes will be studied by 11 institutions. A total of 6600 patients (600 patients in each institution) will be examined; all regimes will be employed by each of the participating institutions on a randomized basis, standard forms for the collection of pertinent clinical information and for the evaluation of the efficiency of each regime have been prepared and will be completed at each institution. Methods for cross evaluations are also included. Statistical analyses will be performed by the Department of Biomathematics at M. D. Anderson Hospital.

Progress: New contract

Significance for Cancer Research (NCP Objective____Approach___)

Project Officer: Dr. William Pomerance
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
[illegible]: Diagnostic Steering Committee
[illegible]:

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues

Principal Investigator: Carlton F. Hazlewood, Ph.D.
Name/Address Baylor College of Medicine
Performing Houston, Texas
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To determine T_1, T_2 and T_1/T_2 in breast lesions. To conduct a blind study on surgical specimens in conjunction with Department of Pathology. To explore the relaxation time in the rotating frame $(T_{1\rho})$, compound relaxation times, temperature dependence of relaxation times, feasibility of using relaxation time in time/detection of cancer surrounded by normal tissue.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Committee
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Cornelius A. Tobias, Ph.D.
Name/Address USAEC, University of California
Performing Lawrence Berkeley Laboratory
Organization: Berkeley, California 94720

Contract Number:
Starting Date: Expiration Date:
Goal: To determine if a proton beam from a high energy (200-400 Mev) source is capable of the improvement in the diagnosis of cancer.

Approach: Using accelerators available at Berkeley, particularly the 184" cyclotron and the Bevalac heavy-ion accelerator, we plan to develop a technique for diagnostic particle radiography of humans. We shall proceed by experimentally testing radiologic properties of protons, helium ions and carbon ions; and of detectors such as photographic film and plastic, nuclear-track-recording films. Human diagnostic studies on cancer will proceed with the particles and detectors that offer optimum resolution and contrast at low diagnostic dose levels. Preliminary studies with accelerated oxygen ions and plastic detectors indicate that this system has superior density resolution for the imaging of soft tissues. It is anticipated that particle radiography may materially aid in detection of small soft-tissue tumors, in delineating the extent of invasive tumors that have different density from normal tissue, and in detecting blood clots inside the body. Diagnosis of soft-tissue abnormalities of the brain and direct studies of the formation of atheromatous plaques in large blood vessels will also be attempted.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: A. R. Moosa, M.D.
Name/Address Performing Organization: University of Chicago, Chicago, Illinois

Contract Number:
Starting Date: Expiration Date:

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5 year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Lester S. Skaggs, Ph.D.
Name/Address
Performing
Organization: University of Chicago
5801 Ellis Avenue
Chicago, Illinois 60637

Contract Number:
Starting Date: Expiration Date:
Goal: To determine if a proton beam from a high energy (200-400 Mev) source is capable of the improvement in the diagnosis of cancer.

Approach: Using accelerators available at Berkeley, particularly the 184" cyclotron and the Bevalac heavy-ion accelerator, we plan to develop a technique for diagnostic particle radiography of humans. We shall proceed by experimentally testing radiologic properties of protons, helium ions and carbon ions; and of detectors such as photographic film and plastic, nuclear-track-recording films. Human diagnostic studies on cancer will proceed with the particles and detectors that offer optimum resolution and contrast at low diagnostic dose levels. Preliminary studies with accelerated oxygen ions and plastic detectors indicate that this system has superior density resolution for the imaging of soft tissues. It is anticipated that particle radiography may materially aid in detection of small soft-tissue tumors, in delineating the extent of invasive tumors that have different density from normal tissue, and in detecting blood clots inside the body. Diagnosis of soft-tissue abnormalities of the brain and direct studies of the formation of atheromatous plaques in large blood vessels will also be attempted.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: C. Ralph Buncher, Sc.D.
Name/Address Performing Organization: University of Cincinnati Medical Center
3223 Eden Avenue
Cincinnati, Ohio 45219

Contract Number:
Starting Date: Expiration Date:

Goal: To collect and analyze data from the three collaborating lung cancer groups in the lung cancer detection program directed to the detection of early lung cancer in high risk patients to see if this will reduce morbidity and mortality.

Approach: Procedure Manual will be prepared and forms will be created so that uniform data will be received by the Statistical Center. The data will be monitored, entered into the computer and analyzed. Reports will be made periodically. Statistics will be analyzed for significance.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis
Site Visit Date: February 26, 19
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Sadek K. Hilal, M.D., Ph.D.
Name/Address Performing Organization:
Columbia University
College of Physicians and Surgeons
630 East 168th Street
New York, NY 10032

Contract Number:
Starting Date: Expiration Date:

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nucleid brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by means of a common protocol and form developed by this group. Patients that will be involved will include patients with definite symptoms suggesting a space occupancy intracranial lesion, patients with a minimal but significant probability of a primary or metabolic brain tumor and patients considered to be normal for the existence of a brain lesion. The number of patients available in this study will provide statistically significant information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Advisory Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: D. Gordon Potts, M.D.
Name/Address Performing Organization: Cornell University Medical College
1300 York Avenue
New York, NY 10021

Contract Number:
Starting Date: Expiration Date:

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nucleid brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by means of a common protocol and form developed by this group. Patients that will be involved will include patients with definite symptoms suggesting a space occupancy intracranial lesion, patients with a minimal but significant probability of a primary or metabolic brain tumor and patients considered to be normal for the existence of a brain lesion. The number of patients available in this study will provide statistically significant information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Advisory Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Conduct of a Tumor Registry for Patients Who Have Had Extensive X-Radiation or Chemotherapy

Principal Investigator: M. Dean Nefzger, Ph.D.
Name/Address Duke University
Performing Durham, NC 27710
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: To define and learn more about the prognosis of patients where disease has been rendered inactive and possibly "cured" by intensive x-radiation or chemotherapy; to establish a group of individuals with a strong possibility of high risk for second cancers and to have them available for accumulation of blood samples that can be used possibly to establish early incidence of recurrent malignant disease or new malignant disease at the same time of determining their prognosis.

Approach: The Contractor will devote the first year to establishing the feasibility of acquiring and maintaining such a registry and its ability to maintain contact with these patients for establishing on a regular basis the status of the patients and on occasion obtaining blood specimens for storage and eventual study. While the original approach may be local (within North Carolina), if feasibility is demonstrated, the geographic area of involvement will be expanded.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 1)

Project Officer: Dr. William Pomerance
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Application and Evaluation of "Xonics" Electron Radiography

Principal Investigator: Luther W. Brady, M.D. Leonard Stanton, M.S.
Name/Address Hahnemann Medical College and Hospital
Performing 230 North Broad Street
Organization: Philadelphia, Pennsylvania 19102

Contract Number:
Starting Date: Expiration Date:

Goal: An evaluation of both present and improved versions of the Xonics x-ray imaging system, in order to report the following information:

1) Basic scientific information for technique design and optimization;

2) Extensive clinical experience to determine the most promising uses of electron radiography, as well as studies where conventional radiography may be preferable; and

3) New approaches to the basic problem of comparing the ability of different imaging systems to demonstrate marginally detectable pathology.

Approach: In order to accomplish these objectives, the Contractor will make basic scientific measurements as to response to x-rays, detail resolution, noise and reliability and reproducibility of clinical equipment. Finally, a clinical comparison and evaluation will be performed involving 16 nationally recognized diagnostic radiologists who are very interested in contributing to the evaluation process. This will include normals (40%) as well as films demonstrating pathology; pairs will not be presented as pairs, nor will each evaluator necessarily receive pairs.

Progress: New Contract

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date: January 22, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator: William D. Odell, M.D., Ph.D.
Name/Address Professional Staff Association
Performing Harbor General Hospital
Organization: Torrance, Los Angeles, California

Contract Number:
Starting Date: Expiration Date:
Goal: To develop hormone markers, now known and not now known, that might indicate the presence of small (early) treatable cancer.

Approach: 1) To use techniques and assays developed in our laboratories to measure ADH, vasotocin, oxytocin, alpha and beta MSH, ACTH, alpha and beta TSH, and alpha and beta HCG to search for tumor elaboration of these peptides among cancer patients; 2) to develop new techniques for separating and easily identifying "big forms" or "prohormone" forms of ACTH, gastrin, and pancreozymin-cholvcystokinin (PZ-CCK); 3) to search for the existence of such big forms of: ADH, oxytocin, alpha and beta MSH, luteinizing hormone releasing hormone, and alpha and beta glycopeptides; 4) to develop peptide assays for fetal hormones related to vasopressin, such as mesotoctin, as well as other fetal peptides and proteins such as fetuin, and to search for their elaboration by cancers; and 5) to use the insights and techniques gained in these studies to identify other nonhormonal peptides which might be used as cancer markers.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Thermography in Mass Screening for Breast Cancer

Principal Investigator: Name/Address Performing Organization:	Raymond Fink, M.D. Health Insurance Plan of Greater New York New York, New York

Contract Number: N01-CN-35-21
Starting Date: June 28, 1973 Expiration Date: June 27, 1975
Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by three methods: clinical examination, thermography and mammography, each modality being evaluated independently. To obtain the study popululation a random sample of all women age 45-65 belonging to medical groups of HIP will be selected. All findings will be reviewed by medical coordinator, who will be responsible for follow-up medical recommendations.

This project is being run in conjunction with Thomas Jefferson University. N01-CN-35-27

Progress: Screening is progressing. No data are available at this time.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Evaluation of a non-radiation detection methodology.

Project Officer: Harvey Geller
Program: Cancer Control Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: **Evaluation of Thermography in Mass Screening for Breast Cancer**

Principal Investigator: Dr. John D. Wallace
Name/Address Thomas Jefferson University
Performing Philadelphia, Pennsylvania
Organization:

Contract Number: N01-CN-35027
Starting Date: June 28, 1973 Expiration Date: June 27, 1975
Goal: To evaluate the contribution thermography may make in increasing the effectiveness and/or efficiency of breast cancer screening programs.

Approach: 20,000 women will be screened by three methods: clinical examination, thermography and xerography, each modality being evaluated independently. To obtain the study population a random sample of all women age 45-65 from a variety of sources will be selected: American Cancer Society screenees, Bell Telephone Company employees, etc. All findings will be reviewed by medical coordinator who will be responsible for follow-up medical recommendation.

This project is being run in conjunction with the Health Insurance Plan of Greater New York. N01-CN-35-21

Progress: Approximately 2,000 women were screened by April 15, 1974. No comparative data are available at this time.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Evaluation of a non-radiation detection method.

Project Officer: Harvey Geller
Program: Cancer Control Site Visit Date:
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer-Early Detection, Localization and Therapy

Principal Investigator: John K. Frost, M.D.
Name/Address Department of Pathology
Performing Johns Hopkins University
Organization: Baltimore, Maryland

Contract Number: N01-CB-92172
Starting Date: June 28, 1969 **Expiration Date:** June 27. 1974

Goal: 1) Develop improved cytologic methods for sputum examination for the early detection of lung cancer; 2) Develop improved methods for the diagnostic localization of lung cancer.

Approach: The project follows a group of chemical and asbestos workers at high risk of lung cancer for sputum cytology studies. These are directed to the biologic significance of the morphology of mild, moderate and severe cell atypia, by correlation with patient follow-up. Patients cytologically suspicious or positive for cancer are studied for better localization techniques, mainly with tantalum dust bronchography and fiberoptic bronchoscopy. Improved equipment is also under development. (See also N01-CN-45037)

Progress: Men exposed to bis-chlorethylether have a rate of lung cancer three times that of cigarette smokers. Data on various methods of inducing and collecting sputum, preparation and examination of specimens is under analysis to determine the best. Tantalum bronchography has been useful, but needs control of particle size and better delivery methods. Fiberoptic bronchoscope has been able to localize every cancer detected by cytology. These early cancers are frequently superficial and "in situ," and sometimes multicentric.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early detection is the best present hope for improving the mortality from lung cancer.

Project Officer: William Pomerance, M.D.
Program: Diagnosis **Site Visit Date:** May 7, 1974
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $500,000

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non Neoplastic Tissues

Principal Investigator:	Donald P. Hollis, Ph.D.
Name/Address	The Johns Hopkins University
Performing	34th and Charles Street
Organization:	Baltimore, MC 21218

Contract Number:
Starting Date: Expiration Date:

Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.

Approach: To carry out measurements of T_1 T_2 and $T_{1\rho}$ on a variety of malignant, normal and diseased but not malignant tissues removed at surgery; these will be carried out blindly in conjunction with the Department of Pathology. There will also be an evaluation of the sensitivity of NMR measurement to small quantities of malignant cells mixed with normal cells.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Committee
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: Patrick J. Fitzgerald, M.D.
Name/Address Performing Organization: Memorial Hospital for Cancer and Allied Diseases
1275 York Avenue
New York, NY 10021

Contract Number:
Starting Date: Expiration Date:

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5 year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer - Early Detection, Localization and Therapy

Principal Investigator:	Myron R. Melamed, M.D.
Name/Address Performing Organization:	Memorial Hospital for Cancer and Allied Diseases 444 East 68th Street New York, NY 10021

Contract Number:

Starting Date: September 1, 1973 Expiration Date: August 31, 1974

Goal: To evaluate sputum cytopathology in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization (e.g., before progression to x-ray positive) and in general the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3 yr. period 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 yrs., all to be followed for at least an additional five year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: New contract

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer: William Pomerance, M.D.

Program: Lung Cancer Diagnosis Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY74 Funds: $529,890

CONTRACT RESEARCH SUMMARY

Title: Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or more (over 7-10 mls per 24 hrs) of Human Blood in Human Feces

Principal Investigator: Curtis L. Songster, M.D.
Name/Address Performing Organization: University of Louisville Foundation, Inc.
Louisville, Kentucky 40201

Contract Number:
Starting Date: Expiration Date:
Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 mls in 24 hrs) in human fecal material by a simple, accurate (98%) examination.

Approach: The Contractor shall develop a procedure for the detection of human blood in feces by utilizing antigens specific for the globin portion (more specifically, Alpha chain) of hemoglobin. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry and fish diets and be truly negative by the proposed test.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Paul F. J. New, M.D.
Name/Address Massachusetts General Hospital
Performing Fruit Street
Organization: Boston, Massachusetts 02114

Contract Number:
Starting Date: Expiration Date:

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nucleid brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by means of a common protocol and form developed by this group. Patients that will be involved will include patients with definite symptoms suggesting a space occupancy intracranial lesion, patients with a minimal but significant probability of a primary or metabolic brain tumor and patients considered to be normal for the existence of a brain lesion. The number of patients available in this study will provide statistically significant information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Advisory Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: Hillier L. Baker, M.D.
Name/Address Mayo Foundation
Performing Rochester, Minnesota
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nucleid brain scan, angiography and pneumoencephalography.
Approach: Five (5) cooperating institutions will evaluate this instrument by means of a common protocol and form developed by this group. Patients that will be involved will include patients with definite symptoms suggesting a space occupancy intracranial lesion, patients with a minimal but significant probability of a primary or metabolic brain tumor and patients considered to be normal for the existence of a brain lesion. The number of patients available in this study will provide statistically significant information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Advisory Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Detection and Localization of Bronchogenic Cancer

Principal Investigator: Robert S. Fontana, M.D.
Name/Address Mayo Clinic
Performing Rochester, Minnesota
Organization:

Contract Number: NO1-CB-22000
Starting Date: August 11, 1973 Expiration Date: Aug. 31, 1974
Goal: 1) Improve methods for the early diagnosis of cancer of the lung. 2) Determine the exact improvement in long time survival of patients with lung cancer due to the application of new techniques for early diagnosis.
Approach: A population of high risk patients (i.e., heavy smokers) who are coming to the Mayo Clinic for symptoms other than lung cancer will be examined thoroughly and then put into one of two groups. The studied group will receive an annual chest x-ray examination and sputum examination three times a year that are mailed in. The control group will have an initial chest x-ray examination and a sputum examination and then it will be suggested that they have an annual chest x-ray done near their home and mailed to the Mayo Clinic. It is hoped (but not provided for) that all patients suspected of lung cancer by cytology or x-ray examination will return to the Mayo Clinic for treatment and possible resection of a small cancer.

Screenees with positive sputum for cancer, but with negative chest x-rays will be admitted for exhaustive studies by means of fiber optic bronchoscopy so that localization of a cancer can be done and followed by a wedge resection or less than a pneumonectomy. Computer analysis of chest x-rays is being studied. Hematoporphyrin for localization of tumors is also being studied.
Progress: A total of 4,353 potential candidates for the Project had been screened as of March 1, 1974. Previously unsuspected lung cancer has been detected in 0.8 percent of eligible candidates who have completed initial screening, and in nine other "participant" subjects during rescreening. Surgical resectability rates have been excellent.

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer: William Pomerance, M.D.
Program: Lung Cancer Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen (CEA) Test, A New Method and Its Evaluation

Principal Investigator: Vay Liang W. Go, M.D.
Name/Address Mayo Clinic
Performing Rochester, Minnesota 55901
Organization:

Contract Number: NO1-CB-23854
Starting Date: June 15, 1973 Expiration Date: June 14, 1975
Goal: To evaluate the carcinoembryonic antigen test in the early diagnosis of carcinoma of the large bowel.

Approach: To develop a new and simpler method of quantitating the blood CEA level and a comparison of these findings with the Hansen test since the Hansen anti sera was found to be the most sensitive as compared to anti sera from Drs. Gold, Todd, Turner, and Rosevear. This test will be used on blood from several hundred cases of gastro-intestinal cancer, lung cancer, normal subjects and others who will have non-cancerous diseases such as inflammatory bowel disease, cirrhosis, etc.

Progress:
Phase I: We have currently completed the collection of blood specimens and the abstracting of the biological data of 3100 patients studied under this section, including 531 healthy subjects, 793 patients with benign gastro-intestinal disorders and 613 patients with benign non-gastrointestinal disorders. All patients who are normal or with benign and malignant disorders have undergone complete standard Mayo Clinic physical examinations and appropriate daignostic procedures were carried out. The results are presently being analyzed statistically. Parts of this body of data have been presented at eight national meetings.

Phase II: As originally planned, we are currently carrying out the projects proposed. Collaborative work has been set up with the Mayo Lung Cancer Project (NCI-CB-22000). Serial blood specimens are obtained from patients with suspected lung cancer as well as with heavy smokers. CEA is determined and the results will be compared to other standard diagnostic procedures such as cytology, chest x-ray, bronchoscopy and other blood studies. Similarly, blood specimens are also obtained in patients who are suspected to have colon cancer who have undergone colonoscopy.
Significance for Cancer Research (NCP Objective__Approach___)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Develop Methods for Detecting Pancreatic Cancer at an Early or Small Stage and Prior to the Presence of Metastasis

Principal Investigator: Van Liang W. Go, M.D.
Name/Address Mayo Foundation
Performing 200 First Street, Southwest
Organization: Rochester, Minnesota 55901

Contract Number:
Starting Date: Expiration Date:

Goal: To evaluate diagnostic methods and findings in patients undergoing abdominal exploration for supposed pancreatic cancer in order to establish a base that can be used for the detection of small pancreatic cancer before metastasis that may lead to an increase in the 5 year survival from 1% now prevalent to 13% or more.

Approach: Three cooperating institutions will study, in a predetermined manner by a common protocol, patients who are suspected of having cancer of the pancreas and are about to undergo abdominal surgery. Special studies delineated in the protocol will include preoperative and operative study of CEA in peripheral and portal venous blood. Special studies of pancreatic excretions obtained through a fiberoptic scope in the duodenum, pancreatic and biliary ducts will be undertaken. Such secretions will be studied for tumor associated antigens, abnormal enzyme content, biochemical alterations, as well as cytological changes which will permit the earlier diagnosis of pancreatic cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of a Simple, Quick, Accurate Method of the Detection of Small Amounts or more (over 7-10 mls per 24 hrs) of Human Blood in Human Feces

Principal Investigator: Richard E. Rosenfield, M.D.
Name/Address Performing Organization: Mount Sinai School of Medicine
New York, N.Y. 10029

Contract Number:
Starting Date: Expiration Date:

Goal: To generate a procedure that will detect small amounts of human blood (over 7-10 mls in 24 hrs) in human fecal material by a simple, accurate (98%) examination.

Approach: The contractor shall develop a procedure for the detection of human blood in feces by utilizing red blood cell surface antigens specific for human blood. Application of the test will be made to an adequate number of persons both known to be bleeding and thought not to be bleeding; an adequate number of controls must have been on high meat, poultry and fish diets and be truly negative by the proposed test.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes that May Presage the Presence of Cancer

Principal Investigator: Eric A. Barnard, Ph.D. Tsann Ming Chu
Name/Address Dept. of Biochemistry Roswell Park Mem. Institute
Performing SUNY at Buffalo 666 Elm Street
Organization: Buffalo, NY 14214 Buffalo, NY 14203
SUNY Research Foundation, P.O. Box 7126, Albany, NY 12224

Contract Number:
Starting Date: Expiration Date:

Goal: To develop and establish a highly sensitive test for a specifically prostatic form of serum acid phosphatase as a marker for early prostatic cancer.

Approach: It is intended to prepare in bulk the isoenzymes of human prostatic acid phosphatase, by methods previously proven in this laboratory in small-scale work. Both the water-extracted types, which are believed to be secreted in the prostatic fluid, and the detergent-extracted type (obtained by a Tween method in previous work) will be separately examined. Each will be used as an antigen to obtain a radio-immunoassay method, for its specific detection at very low levels in sera. Each will also be studied for any specific binding or inhibitory property that can characterize it at low levels in the presence of other phosphatases.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Nuclear Magnetic Resonance to be Studied in Neoplastic and Non-Neoplastic Tissues

Principal Investigator: Raymond Damadian, M.D.
Name/Address Downstate Medical Center
Performing Brooklyn, New York
Organization: SUNY Research Foundation, P.O. Box 7126, Albany, NY 12224

Contract Number:
Starting Date: Expiration Date:
Goal: The use of nuclear magnetic resonance spectroscopy for the identification of malignant, neoplastic tissue so that this tissue can be differentiated from normal and non-neoplastic, diseased tissue.
Approach: To blindly study approximately 200 surgical specimens by means of NMR determinations. To determine the extent to which malignant tissue can be detected by NMR in the presence of normal tissue. To explore the feasibility of detecting internal tumors in small laboratory animals.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Committee
Relevance Review Group: Diagnosis Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Proposal for Thermography Technologist Training Program

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address University of Oklahoma
Performing Health Sciences Center
Organization: College of Medicine, P.O. Box 26901
Oklahoma City, Oklahoma 73190

Contract Number:
Starting Date: Expiration Date:

Goal: To train thermography technicians to improve the quality of thermograms obtained in breast examination in order to increase our ability to demonstrate early cancer.

Approach: It is proposed to establish a training program for thermographic technicians involved in the A.C.S.-N.C.I. Breast Cancer Demonstration Projects. The course would run for one week, and no more than two technicians would be scheduled in any one week. This will provide teaching on a one-to-one basis. Audio-tape slides will be available as part of the curriculum. The trainee will spend approximately half of each day with the University of Oklahoma technicians in the screening center and will observe techniques and eventually carry them out. Conferences with the Breast Screening physicians, bioengineers and technologists will be arranged to provide an opportunity for questions.

It is expected that a method of evaluating the effectiveness of the course will be developed. There will also be available a thermographic technique book identifying the various technical problems to be encountered and their resolution.

Study carrels for the audio-tape slide lectures will be available.

Progress: New Contract

Significance for Cancer Research (NCP Objective___Approach___)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Steering Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development of Methods of Bowel Preparation Preparatory to Barium Enema or Colonoscopy

Principal Investigator: Harry W. Fischer, M.D.
Name/Address
Performing
Organization: University of Rochester
School of Medicine and Dentistry
Rochester, NY 14642

Contract Number:
Starting Date: Expiration Date:
Goal: To perfect methods for insuring a clean colon for the benefit of the radiologist and/or the endoscopist. The technique should not require long periods of time (within a 24 hr period) and without excessive dietary restrictions and without excessive patient discomfort or inconvenience.

Approach: To put to trial combinations of some older methods, or combinations of certain features of older methods, along with the testing of a previously little used method of assisting conversion of solid stool to semisolid or liquid phase. It is proposed that fecal material be converted to a more liquid form (Embring). and the patient then be assisted to evacuate. The entrance of additional material into the colon from above would be minimized by a certain degree of dietary restriction prior to the definitive portions of the technique aimed at stool conversion and evacuation.

Progress: New contract

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Biochemical Markers or Enzyme Changes that May Presage the Presence of Cancer

Principal Investigator: Joshua Lederberg, Ph.D.
Name/Address Stanford University School of Medicine
Performing Stanford, CA 94305
Organization: Board of Trustees, Leland Stanford, Jr., University
Stanford, CA 94305

Contract Number:
Starting Date: Expiration Date:

Goal: To develop further the uses of gas-liquid chromatography and mass spectometry instrumentation, under computer management, for the study of derangements of human biochemical metabolism due to neoplastic change; this to be done for the purpose of identifying new metabolites, or altered levels of known metabolites, which might be diagnostic of cancer.

Approach: (a) To screen urine by GS/MS from control subjects and from individuals suffering from the following forms of cancer: (1) prostatic cancer, (2) bladder cancer, (3) Hodgkin's disease, and (4) various lymphomas.

(b) The quantitation of urinary beta-aminoiso-butyric acid levels from cancer patients.

(c) The quantitation of urinary protein amino acid levels in cancer patients.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of the Use of Computerized Transaxial Tomography in the Diagnosis of Brain Tumors

Principal Investigator: David O. Davis, M.D.
Name/Address
Performing
Organization:
George Washington University
Rice Hall - 6th Floor
Washington, D. C. 20006

Contract Number:
Starting Date: Expiration Date:

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nucleid brain scan, angiography and pneumoencephalography.

Approach: Five (5) cooperating institutions will evaluate this instrument by means of a common protocol and form developed by this group. Patients that will be involved will include patients with definite symptoms suggesting a space occupancy intracranial lesion, patients with a minimal but significant probability of a primary or metabolic brain tumor and patients considered to be normal for the existence of a brain lesion. The number of patients available in this study will provide statistically significant information.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 5)

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Radiology Advisory Committee
Relevance Review Group: Diagnostic Steering Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Arthur J. Present, M.D.
Name/Address University of Arizona
Performing Arizona Medical Center
Organization: Tuscon, Arizona 35732

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Myron Moskowitz, M.D.
Name/Address University of Cincinnati College
Performing of Medicine
Organization: Cincinnati, Ohio

Contract Number: N01-CN-35024
Starting Date: June 29, 1973 Expiration Date: June 28, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 3209 to March 30, 1974. 24 biopsied: 9 in situ, 6 invasive with positive nodes and 9 invasive with negative nodes.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Richard G. Lester, M.D.
Name/Address Duke University
Performing Durham, NC 27710
Organization:

Contract Number: NO1-CN-45064
Starting Date: February 15, 1974 Expiration Date: February 14, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $130,354

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator:	Harold J. Isard, M.D.	Marc S. Lapayowker, M.D.
Name/Address	Albert Einstein Med. Ctr.	Temple University Hosp.
Performing	York and Taber Roads	3401 North Broad Street
Organization:	Philadelphia, PA 19141	Philadelphia, PA 19140

Contract Number: N01-CN-45058
Starting Date: January 2, 1974 Expiration Date: January 1, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 709 to March 15, 1974.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $141,222

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator:	R. Waldo Powell, M.D.	A. Hamlin Letton
Name/Address	Emory University	Georgia Baptist Hospital
Performing	Atlanta, GA 30322	Atlanta, GA 30312
Organization:		

Contract Number: NO1-CN-35016
Starting Date: May 9, 1973 Expiration Date: May 8, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 666 to March 15, 1974 - Emory University
Screened 2270 to March 15, 1974 - Georgia Baptist Hospital

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: William L. Donegan, M.D.
Name/Address Ellis Fischel State Cancer Hospital
Performing Columbia, Missouri 65201
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Margaret Abernathy, M.D.
Name/Address Georgetown University
Performing 3800 Reservoir Road
Organization: Washington, D.C. 20007

Contract Number: NO1-CN-45062
Starting Date: February 7, 1974 Expiration Date: February 6, 1974
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 74 Funds: $135,318

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cance

Principal Investigator: Morton J. Goodman
Name/Address Good Samaritan Hospital and
Performing Medical Center
Organization: Portland, Oregon 97201

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Philip Strax, M.D.
Name/Address Guttman Breast Institute
Performing New York, NY
Organization:

Contract Number: NO1-CN-35004
Starting Date: April 18, 1973 Expiration Date: April 17, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: 10,000 patients have been screened. 133 biopsies have been done with diagnosis of 48 cancers (36%). Eight of the cancers were positive only with mammography.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Donald C. Young, M.D.
Name/Address Iowa Lutheran Hospital
Performing University and Penn Streets
Organization: Des Moines, IA 50316

Contract Number: N01-CN-45067
Starting Date: February 27, 1974 Expiration Date: February 26, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $122,366

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Loren J. Humphrey, M.D., Ph.D.
Name/Address Performing Organization: Kansas University Medical Center
Kansas City, Kansas

Contract Number: NO1-CN-35026
Starting Date: June 19, 1973 Expiration Date: June 18, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Condict Moore, M.D.
Name/Address University of Louisville
Performing Louisville, Kentucky
Organization:

Contract Number: N01-CN-35012
Starting Date: April 17, 1973 Expiration Date: April 16, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 2300 to March 15, 1974.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Thomas Carlile, M.D.
Name/Address Virginia Mason Hospital
Performing Seattle, Washington
Organization:

Contract Number: NO1-CN-35028
Starting Date: June 15, 1973 Expiration Date: June 14, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 1841 to March 15, 1974.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator:	Robert Schweitzer, M.D.
Name/Address	Samuel Merritt Hospital
Performing	384 34th Street
Organization:	Oakland, CA 94609

Contract Number: N01-CN-45068

Starting Date: February 25, 1974 Expiration Date: February 24, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.

Program: Cancer Control Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY74 Funds: $142,007

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Walter M. Whitehouse, M.D.
Name/Address University of Michigan
Performing University Hospital
Organization: Ann Arbor, MI 48104

Contract Number: N01-CN-45049
Starting Date: March 18, 1974 Expiration Date: March 17, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Dr. William Pomerance
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $133,333

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Alfred M. Popma, M.D.
Name/Address Mountain States Tumor Institute
Performing Boise, Idaho
Organization:

Contract Number: N01-CN-35026
Starting Date: June 25, 1973 Expiration Date: June 24, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 1740 to March 15, 1974

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Benjamin Rush
Name/Address College of Medicine and Dentistry
Performing of New Jersey
Organization: Newark, New Jersey

Contract Number: N01-CN-35006
Starting Date: June 29, 1973 Expiration Date: June 29, 1975

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Fund:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: JoAnn D. Haberman, M.D.
Name/Address Performing Organization: University of Oklahoma
Oklahoma City, Oklahoma

Contract Number: N01-CN-35020
Starting Date: June 18, 1973 Expiration Date: June 17, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened approximately 5,000 to March 15, 1974

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Fred I. Gilbert, Jr., M.D.
Name/Address Pacific Health Research Institute
Performing Alexander Young Building
Organization: Honolulu, HI 96813

Contract Number: NO1-CN-45046
Starting Date: December 19, 1973 Expiration Date: December 18, 1974

Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress:

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $128,340

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Bernard Fisher, M.D.
Name/Address University of Pittsburgh
Performing 3550 Terrace Street
Organization: Pittsburgh, PA 15261

Contract Number: N01-CN-45065
Starting Date: February 15, 1974 Expiration Date: February 14, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds: $135,762

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Herbert P. Constantine, M.D.
Name/Address Rhode Island Hospital
Performing Providence, RI 02902
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Duncan L. Moore, M.D.
Name/Address St. Joseph's Hospital
Performing Houston, Texas 77002
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Marvin V. McClow, M.D.
Name/Address St. Vincent's Medical Center
Performing Jacksonville, Florida
Organization:

Contract Number: N01-CN-35008
Starting Date: June 20, 1973 Expiration Date: June 19, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 706 to March 15, 1974

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Lewis W. Guiss, M.D.
Name/Address University of Southern California
Performing University Park
Organization: Los Angeles, California 90007

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: John R. Amberg, M.D.
Name/Address Vanderbilt University Hospital
Performing Nashville, Tennessee 37232
Organization:

Contract Number:
Starting Date: Expiration Date:
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: Leslie W. Whitney, M.D.
Name/Address Performing Organization: Wilmington Medical Center
Wilmington, DE 19805

Contract Number: N01-CN-45047
Starting Date: January 1, 1974 Expiration Date: December 31, 1973
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group:
FY74 Funds: $130,652

CONTRACT RESEARCH SUMMARY

Title: Demonstration Project in Earlier Detection of Breast Cancer

Principal Investigator: James E. Youker, M.D.
Name/Address The Medical College of Wisconsin
Performing Milwaukee, Wisconsin
Organization:

Contract Number: N01-CN-35018
Starting Date: June 18, 1973 Expiration Date: June 17, 1975
Goal: To have the American Cancer Society through its volunteers recruit 5000 women in the first year and an additional 5000 in the second year to come to a project of thermography, mammography, and physical examination for the detection of breast cancer and be rescreened annually for 5 screenings and to be followed annually for 5 years thereafter.

Approach: Each of 27 projected projects (29 institutions) funded by the American Cancer Society and the National Cancer Institute will work with their local cancer society (in a variety of ways, some hopefully unique) so that women without symptoms relative to their breasts will be examined for breast cancer by means of mammography, thermography, and physical examination so that nonpalpable and "early" breast cancer without axillary node metastasis can be detected and treated and prolong life free of breast cancer and even hopefully to "cure" breast cancer.

Progress: Screened 3004 to March 15, 1974.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: William Pomerance, M.D.
Program: Cancer Control Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Breast Cancer Demonstration Projects.

Principal Investigator: Name/Address Performing Organization:	Dr. James Gumnick University City Science Center 3508 Science Center Philadelphia, Pennsylvania 19104

Contract Number: No 1-CN-35035

Starting Date: June 28, 1973 Expiration Date: June 27, 1974

Goal: To operate a Data Management Center for the Breast Cancer Demonstration Projects.

Approach: 1) Design, implement, and maintain a computer system for the collection, editing, storage, and retrieval of all data collected in the Demonstration Project. 2) Assume the responsibility for the receipt and control of all documents and other materials transmitted to the Data Management Center by the Demonstration Projects, the Data Conversion Centers, the Computer Center, NCI, ACS, and any other institution forwarding patient data to the Data Management Center. 3) Assure the completeness and accuracy of all records with manual and computer edit procedures. 4) Maintain records, provide listings, provide reports, provide manuals, etc., etc., to fulfill its function as a Data Management Center.

Progress: During this past year the Center has worked with the Project Officer to establish forms and distribute them. Personnel has worked with the Project Directors and their staffs to establish the operation and flow of data to fulfill the responsibilities of the Data Management Center.

Significance for Cancer Research (NCP Objective____Approach____)

Project Officer: Dr. William Pomerance Mr. Theodore Weiss

Program: Cancer Control Site Visit Date: Jan. 10, 1974

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY74 Funds: $495,000

CONTRACT RESEARCH SUMMARY

Title: Study of Intrabronchial Deposition of Inhaled Radioopaque Particles

Principal Investigator: Morton Lippmann, Ph.D.
Name/Address Institute of Environmental Medicine
Performing New York University Medical Center
Organization: 550 First Avenue
New York, N.Y. 10016

Contract Number: N01-CB-33855
Starting Date: November 1, 1972 Expiration Date: September 30, 1974
Goal: To determine the amount of tantalum dust that must be deposited for satisfactory bronchography.

Approach: Using casts of the bronchial tree, tantalum is deposited in various amounts and ways, and the adequacy of deposition assessed by x-rays and other methods. Rates of deposition and distribution from dust clouds with varying particle size and concentration are evaluated in relation to airway dynamics, subject position, and so forth.

Progress: It has been shown in models that optimal bronchography requires about 8 mg/cm^2 of tantalum. This deposition can be produced with dust averaging particles 3 microns in diameter, using a dust generator developed at the University of Pittsburgh, a flow rate of 8 L/min and exposure of 30 min prone and 30 min supine. (Generator modification may permit shorter exposure.) The next step will be the determination in humans of the amount of alveolar deposition (an undesirable side effect) and rate of its clearance, not with tantalum, but with a tagged ferric oxide aerosol of equivalent size.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Successful development of tantalum bronchography will greatly improve the diagnostic localization of lung cancer.

Project Officer: I. C. Plough, M.D.
Program: Diagnosis Site Visit Date:
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $10,000

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: John K. Frost, M.D.
Name/Address Department of Pathology
Performing Johns Hopkins University
Organization: Baltimore, Maryland

Contract Number: N01-CN-45037
Starting Date: September 1, 1973 Expiration Date: July 15, 1974

Goal: To determine the effect of the early detection of lung cancer by sputum cytology followed by surgical removal on long-term survival in the disease.

Approach: The study population will be males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment from individuals responding to advertisement, it is planned to accumulate over several years two groups of 4000 each. The test group will receive sputum cytologic examination and chest x-ray every four months; the control group will have an annual chest x-ray, present good medical practice. Subjects suspicious or positive for cancer will be carefully worked up, including fiberoptic bronchoscopy, and cancers will be removed surgically. All subjects will be followed for five years to determine actuarial survival. (See also N01-CB-92172).

Progress: Partial renovation of facilities was completed, and screening of subjects started in January 1974. By April over 200 subjects had been screened. No cancers have been found in this group.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: William Pomerance, M.D.
Program: Diagnosis Site Visit Date: June 1973
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $629,652 (Cancer Control)

CYTOLOGY AUTOMATION CONTRACT PROGRAM

ANNUAL REPORT SUMMARY

July 1, 1973 to June 30, 1974

The Committee on Cytology Automation was established in July, 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation in operational terms for National Cancer Institute's contractual support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Committee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

Three contracts were continued in FY 1974 from previous years. All three contracts emphasized instrument development in categories three and four above. These instruments being evaluated include a multiparameter cell analysis and sorting system at Los Alamos Scientific Laboratory of Atomic Energy Commission (zero resolution); a high-resolution image processing system at the University of Chicago and an intermediate-resolution flow analysis system at the University of Rochester.

Major expansion of the Cytology Automation program in FY 1974 was in areas one and two above with the funding of eight new contracts to investigate the problems associated with preparation and stoichiometric staining of clinical specimens. This area has consistently been the stumbling block on which technically excellent instruments have failed in clinical application. The contracts funded include both development of new techniques and application of techniques from other areas such as tissue culture to the specific problems presented by clinical cytopathologic specimens. Further details on these contracts are given in the individual Contract Research Summaries.

In order to evaluate the biological requirements for a successful automated system, a contract has begun at the University of Chicago to quantify the numbers and types of cells present on cytologic smears from endocervix, ectocervix and vagina (areas one and two above). The data provided by this study will provide a foundation on which to evaluate the compatibility of automated system specimen preparation with preservation of diagnostic value of the specimen.

Finally, the Cytology Automation program has funded contractual support to ensure the investigators working in the program an adequate supply of clinical material with which to test techniques and instruments.

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Stains and Other Optical Markers and New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathology Specimens.

Principal Investigator: Seymour West, Ph.D.
Name/Address University of Alabama
Performing Birmingham, Alabama
Organization:

Contract Number:
Starting Date: Expiration Date:

Goal: Evaluate the applicability of biophysical instrumental analysis to clinical cytopathologic pre-screening.

Approach: The Contractor will apply considerable general experience with biophysical techniques to the evaluation of the possible usefulness of these techniques in clinical cytopathologic pre-screening. These techniques employ acridine orange as a molecular probe and evaluation includes absorption and emission spectra, circular dichroism, optical rotatory dispersion. Preliminary investigation (feasibility study) of lectin-agglutination of clinical specimens will also be performed.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Identification of new machine sensible properties suitable for distinguishing neoplastic from non-neoplastic human cells.

Project Officer: Chester J. Herman, M.D.
Program: Cytology Automation Site Visit Date: 1/22/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $350,379

CONTRACT RESEARCH SUMMARY

Title: **Evaluation of Information Requirements for Image Processing of Cell Samples as Basis for Development of Automated Cell Recognition System.**

Principal Investigator: R. George L. Wied, M.D.
Name/Address Department of Obstetrics and Gynecology
Performing The University of Chicago
Organization: 5801 South Ellis Avenue
Chicago, Illinois 60637

Contract Number: NO1-CB-33873
Starting Date: 5/31/73 Expiration Date: 4/30/76

Goal: Automation of clinical cytologic screening and diagnosis, focussing on definition of information requirements and criteria for high throughput, high resolution image processing-based decision making.

Approach: The Contractor shall evaluate information requirements for characterization and recognition of normal, premalignant and malignant cells from the human female genital tract by means of digital image processing and computer feature extraction applied to high resolution, multi-color digital images of clinical specimens. The cytologic material will include 15 main categories with at least 100 cells each, drom different subjects, so as to include the effects of intra- and inter-individual variation, physiological age, pregnancy, viral infection, and specific and non-specific inflammatory reactions. Robustness of cytologic features and discrimination logic will be assessed as a function of minor variations in staining and fixation procedures.

Progress: Unequivocal cells from gynecologic smears were first scanned monochromatically, digitized, recorded, interactively edited to eliminate extra cellular material and delineate nuclei. For a pilot study, 100 cells of 12 of the 15, enumerated categories were included in a classical "machine learning" experiment using pairwise linear discriminant functions of simple quantitative computer-generated cell features. Completion of the classical test phase was deferred, pending development of an ad hoc hierarch of partially redundant multi-category discriminants. Although unoriginal and problem-specific, this alternative logic is very efficient and highly promising. A new custom rapid, high resolution three color Zerss smp has been interfaced to operator and computer controls and recording media. Performance tests and minor modifications are being made. A second pilot study of 200 squamous cells showed color alone to be a reliable atuomated index of cell maturity.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
A rapid, high-resolution, multi-spectral digital microscanner for clinical cynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the population at large and for the population at high risk.

Project Officer: Judith M.S. Prewitt
Program: Cutology Automation Site Visit Date: 11/20/72
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: DCBD Contract Review Board
FY74 Funds: $183,955

CONTRACT RESEARCH SUMMARY

Title: Studies and Investigations of the Diagnostic Value of Gynecologic Cytopathology Samples.

Principal Investigator:	Marluce Bibbo, M.D.
Name/Address	5841 Maryland Avenue
Performing	University of Chicago
Organization:	Chicago, Illinois 60637

Contract Number:
Starting Date: Deferred **Expiration Date:**
Goal: To determine the cellular content of cytologic samples from vagina, ectocervix and endocervix of patients with various clinical conditions

Approach: The principal investigator will use the large volume of Pap smears available at the University of Chicago, which have been collected over the past 18 years, for a retrospective study of the cellular content of the smears. The smears will be examined and counted for relative numbers of epithelial and inflammatory cells of all types from normal through invasive carcinoma cases. It will also be noted if the cells appear singly or as part of a cluster. The classification will be according to the World Health Organization Plan.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
It is essential in designing an automated screening system to know the content of the samples to be screened.

Project Officer: Mary Cassidy
Program: Cytology Automation **Site Visit Date:** 2/20/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: Approximately $70,000

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Approaches to the Problem of Markers Applicable to Gynecologic Cytopathology Specimens.

Principal Investigator: Professor Torbjorn Caspersson
Name/Address Performing Organization: Institute for Medical Cell Research and Genetics
Nobel Medical Institute
Karolinska Institutet
S-104 01 Stockholm 60, Sweden

Contract Number:
Starting Date: Expiration Date:

Goal: Automation of clinical cytologic screening and diagnosis, concommitant with improvement in diagnostic accuracy and throughput.

Approach: Dr. Caspersson has pioneered the development of innovative methods of investigative cytology and cytogenetics. Under this contract, he will develop and evaluate prototype machines for automatically measuring cytologic features of clinical gynecologic specimens using high resolution scanning microscopy with absorption ultraviolet-spectrophotometry, interferometry and cytofluorometry, individually and in combination. The objectives are (1) an optimal automated, rapid and reliable triple or dual imaging modality instrument for simultaneous assessment of total cellular nucleic acid, DNA, RNA and mass, and corresponding features of gynecologic specimens usually categorized as normal, pre-malignant, carcinoma in situ and invasive carcinoma, using these features.

Progress: This contract has just commenced.

Significance for Cancer Research (NCP Objective 5 Approach 5.3) Development of very rapid high resolution multiple modality automated scanning microscopes will advance image processing approaches for cytologic screening and diagnosis, and promote better understanding of normal, pre-cancerous and cancerous states.

Project Officer: Judith M.S. Prewitt
Program: Cytology Automation Site Visit Date: 2/27/74
Technical Review Group: Committee for Cytology Automation
Relevance Review Group: DCBD Contract Review Board
FY74 Funds:

CONTRACT RESEARCH SUMMARY

Title: Development and Evaluation of New Stains and Other Optical Markers Useful for Cytopathologic Specimens in Suspension.

Principal Investigator: Brian H. Mayall, M.D.
Name/Address Lawrence Livermore Laboratory
Performing Biomedical Division
Organization: Livermore, California

Contract Number:
Starting Date: Expiration Date:

Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: Utilizing their unique combination of flow-sorting and image processing expertise, the investigators will evaluate specimen preparation and staining techniques on clinical samples. The ability to evaluate specimens at both zero and medium to high resolution will assure the mutual compatibility of preparative techniques and safeguard the diagnostic value of samples prepared for automated systems.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Development and evaluation of techniques to rapidly screen cell populations for pre-malignant and malignant cells.

Project Officer: Chester J. Herman, M.D.
Program: Cytology Automation Site Visit Date: 2/1/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $331,670

CONTRACT RESEARCH SUMMARY

Title: **Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopatholody Specimens in Suspension.**

Principal Investigator:	Leopold G. Koss, M.D.
Name/Address	Montefiore Hospital and Medical Center
Performing	111 East 210th Street
Organization:	Bronx, New York 10467

Contract Number:

Starting Date: Deferred Expiration Date:

Goal: To obtain monodisperse cell suspensions from gynecologic cytopathology specimens for use in flow-through systems being developed for cytology automation.

Approach: The principal investigator will study the natural cell attachments and various ways of disrupting these attachments. Electron microscopy will be used to evaluate the effectiveness of the cell attachment (chiefly desmosome) disruption.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy

Program: Cytology Automation Site Visit Date: 2/7/74

Technical Review Group: Ad Hoc Committee

Relevance Review Group: Cancer Diagnosis Steering Committee

FY74 Funds: Approximately $174,158

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Methods for Obtaining Monodisperse Cell Preparations of Gynecologic Cytopathologic Specimens in Suspension and on Slides.

Principal Investigator: Walter E. Tolles, Ph.D.
Name/Address
Performing
Organization: State University of New York
Downstate Medical Center
450 Clarkson Avenue
Brooklyn, New York 11203

Contract Number:
Starting Date: Deferred
Expiration Date:

Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automateion.

Approach: The emphasis will be on lytic and enrichment techniques. Chemical and enzymatic agents will be used to break up clusters as well as mechchanical and ultrasonic shearing techniques. Rate-zonal sedimentation will be used as an enrichment technique.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)

Project Officer: Mary Cassidy
Program: Cytology Automation
Site Visit Date: 2/8/74
Technical Review Group: Ad Hoc Committee
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: Approximately $98,485

CONTRACT RESEARCH SUMMARY

Title: Develop and Evaluate New Stains and Other Optical Markers Useful for Clinical Cytopathological Specimens in Suspension.

Principal Investigator:	Robert C. Leif, Ph.D.
Name/Address	Papanicolaou Cancer Research Institute
Performing	Miami, Florida
Organization:	

Contract Number:
Starting Date: Expiration Date:

Goal: Investigation of new markers and techniques of specimen preparation for clinical cytopathologic screening.

Approach: The investigator will extend the technique of centrifugal cytology to produce improved specimens for analytic instrumentation. Techniques for dialysis staining, heavy metal chelating fluorochromes, and immunofluorescence will be investigated as preliminary feasibility studies.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The development of new markers and preparative techniques for clinical specimens will improve the prospects for more rapid automation of clinical cytopathologic pre-screening.

Project Officer: Chester J. Herman, M.D.
Program: Cutology Automation Site Visit Date: 3/11/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $250,000

CONTRACT RESEARCH SUMMARY

Title: Immunological, Enzymatic and Other Biochemical Markers for Detection of Abnormal Cervical Cells on Slides or in Suspensions by Optical Methods.

Principal Investigator: B.D. Halpern, Ph.D.
Name/Address Performing Organization: Polysciences, Inc. Warrington, Pennsylvania

Contract Number:
Starting Date: Expiration Date:

Goal: Chemical Synthesis and preliminary testing of markers for clinical specimens.

Approach: The Contractor will apply his extensive experience in biochemical synthesis to produce absorptive and flourescent dyes to stoichiometrically quantitate macromolecules, enzymatic activity and antigen-antibody systems suggested by basic scientific research to be of potential utility in distinguishing human malignant and pre-malignant cells from non-neoplastic cells in automated cytopathologic screening instruments.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)

Chemical synthesis and verification of stoichiometric stains useful for clinical use in screening cytopathology specimens.

Project Officer: Chester J. Herman, M.D.
Program: Cytology Automation Site Visit Date: 1/25/74
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $152,988

CONTRACT RESEARCH SUMMARY

Title: Investigation of a Slit-Scan Technique as a Basis for an Automated Prescreening System for Cancer Detection in Cytology.

Principal Investigator: Leon L. Wheeless, Jr. Ph.D.
Name/Address Performing Organization: University of Rochester
School of Medicine and Dentistry
Rochester, New York 14642

Contract Number: NO1-CB-33862
Starting Date: 3/19/74 Expiration Date: 3/18/75
Goal: Automation of clinical cytologic screening and diagnosis

Approach: The investigator will concentrate his effort in the second year on additional biologic studies of sample preparation and cell behavior in flow-through systems. Work done will be evaluated as to its effectiveness on the slit-scan instruments developed in year one. Studies in specimen preparation will include cell dispusion, mucus dissolution, recognition of artifacts and cell orientation and dynamic behavior in sheath flow systems. Work will continue on the slit-scan technique of cell recognition.

Progress: To date work has been done in the area of sample collection, preparation and suspension-staining of clinical cytologic material. Indications are that this area needs to be developed more thoroughly. Progress has been rapid with the use of the cytofluorimeter for identification of normal and abnormal cells. The newly developed slit-scan flow-system is being tested.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed and availability in screening and diagnosis of cancerous and pre-cancerous lesions.

Project Officer: Mary Cassidy
Program: Cytology Automation Site Visit Date: Deferred
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: $396,333

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation.

Principal Investigator:
Name/Address
Performing
Organization:

Jerry Shulman, M.D., M.P.H.
Temple University Medical School
and Hospital
3401 North Broad Street
Philadelphia, Pennsylvania

Contract Number:
Starting Date: Deferred
Expiration Date:

Goal: Evaluation of multiparameter cell sorter designed for cytology automation.

Approach: The Contractor shall deliver to the Laboratory of Pathology 1500 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.4)
The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary Cassidy
Program: Cytology Automation
Site Visit Date: none
Technical Review Group: Committee on Cytology Automation
Relevance Review Group: Cancer Diagnosis Steering Committee
FY74 Funds: Deferred

CONTRACT RESEARCH SUMMARY

Title: Automated Cancer-Cell Sorting and Analysis

Principal Investigator: Dr. J.A. Steinkamp
Name/Address U.S. Atomic Energy Commission
Performing Los Alamos Scientific Laboratory
Organization: Los Alamos, New Mexico 87544

Contract Number: CB-10055
Starting Date: 4/1/74 Expiration Date: 3/31/75
Goal: Automation of Clinical Cytologic Screening and Diagnosis.

Approach: A multiparameter sorter has been developed and is being tested by the Contractor to measure and analyze cytobiological parameters related to malignancy. With both model tumor lines and human squamous specimens, this instrument has separated successfully samples which have as few as eight percent tumor cells on the basis of DNA content alone. In addition to the parameters and combination of parameters currently being investigated, the Contractor's laboratory will develop new sample preparation and staining techniques suitable for analysis by the sorter, will conduct a preliminary study of quantitative immunoflourescence with Herpes II virus assay as a specific indication of malignancy, will investigate lectin binding to surfaces of normal and abnormal squamous cells, will improve the sorter currently at the NCI, and will investigate light scattering and time-of-flight properties of squamous cells in gynocological material. All investigations will be designed and evaluated on their ability to achieve the goal of automating gynecological screening.

Progress: The Contractor has developed a multiparameter cell sorter and has demonstrated that this unit can separate tumor cells from hematopoietic and squamous cells. In addition to the prototype instrument, two improved cell sorters have been constructed. One of these instruments has been installed at the NCI Laboratory of Pathology and the second remains at the Contractor's laboratory. All these instruments are currently used for investigations of clinical cytologic material.

Significance for Cancer Research (NCP Objective 5 Approach 5.3)
The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.
Project Officer: B.J. Fowlkes
Program: Cytology Automation Site Visit Date: Jan. 18, 1973
Technical Review Group: Cytology Automation Committee
Relevance Review Group: DCBD Contract Review Board
FY74 Funds: $335,800

CONTRACT RESEARCH SUMMARY

Title: Maintenance of a Tissue Library and the Processing of Microscopic Slides (NCI 517)

Principal Investigator: Dr. L. B. Thomas

Name/Address
Performing
Organization: Baker Histology Laboratory, P. O. Box 48,
Great Falls, Virginia 22066

Contract Number: NIH-NCI-G-73-3863

Starting Date: December 16, 1972 Expiration Date: December 15, 1973 (Extended to June 15, 1974)

Goal: The contract provides histological sections of a routine nature to the investigators of the National Cancer Institute and maintains a tissue block library to file, store and retrieve tissue embedded paraffin blocks.

Approach: The contract relieves the Pathological Technology Section of the overflow of routine preparation of histological slides of large screening experiments where quality work is not necessary. It also provides 500 square feet of space for storage of blocks.

Progress: Contract has been in effect since December 16, 1972 and as of March 6, 1974 there have been 16,251 H & E slides delivered.

Significance for Cancer Research: (NCP Objective 3 Approach 3.) This contract provides support for Objective 3, by supplying aid to the Pathological Technology Section in lieu of the loss of personnel and space of this Section.

Project Officer: Betty J. Sanders
Program: Cancer Biology Support Site Visit Date: Oct. 12 and 17, 1972

Technical Review Group: Dr. Gazdar, Miss Bowling, Miss Sanders, Dr. Peck, Dr. Thomas and Dr. Dawe
Relevance Review Group: Dr. Berlin, Dr. Plough, Dr. Ketcham, Dr. Thomas, Dr. Lutzner and Mr. Reynolds

FY74 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. W. Bruce Ramsay
Name/Address Computer Services Division, Bldg. 101/A-225
Performing National Bureau of Standards
Organization: Gaithersburg, MD 20234

Contract Number: Y01-CB-80047
Starting Date: 7/1/73 Expiration Date: 6/30/74
Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.
Approach:

N/A

Progress:

N/A

Significance for Cancer Research (NCP Objective____Approach____)

N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support Site Visit Date: N/A
Technical Review Group: Ad hoc
Relevance Review Group:
FY74 Funds: $55,000.

4

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator: Andrew S. Tegeris
Name/Address 9705 N. Washington Blvd., Laurel, Maryland 20810
Performing Organization: Pharmacopathics Research Laboratories, Inc.

Contract Number: N01-CB-33887
Starting Date: July 1, 1973 **Expiration Date:** June 30, 1974

Goal: (1) Develop the means to reduce the effectiveness of external agents for producing cancer by examining response of the pleura of rats to variety of types of asbestos and other durable fibers.

Approach: (4) Improve animal models of human cancer and evaluate effects of combined factors by assessing the response of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: We are currently testing carcinogenic response to many types of durable fibers in the pleura of the rat. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Project Officer: Mearl F. Stanton, M.D.
Program: Cancer Biology Support Site Visit Date: Monthly
Technical Review Group: Ad hoc Relevance Review Group
Relevance Review Group: DCBD Contract Review Board
FY74 Funds: $88,000

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia (NCI-538)

Principal Investigator: John C. Harshbarger, Ph.D.
Name/Address Director, Registry of Tumors in Lower Animals
Performing The Smithsonian Institution
Organization: Washington, D.C. 20560

Contract Number: PH 43-65-1072
Starting Date: July 1, 1973 **Expiration Date**: June 30, 1974
Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.
Approach: The Principal Investigator directs the operation of a Registry of Tumors in Lower Animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made.

Progress: Specimen accessions now number 928, 47 percent of these confirmed as neoplastic. The majority of neoplasms were in teleost fishes, reptiles, and amphibians, with relatively few in 2 invertebrate phyla, the molluscs and arthropods. During the past year several examples of neoplasms from apparently epizootic or enzootic foci were received and classified. The Registry collaborates in investigations seeking to find environmental agents that may be causative factors in these epizootics.

Significance for Cancer Research (NCP Objective 1 Approach 1,4)
Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.
Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support **Site Visit Date**:
Technical Review Group:
Relevance Review Group: DCBD Contract Review Board
FY74 Funds: $82,769

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